METHODS AND REAGENTS FOR THE DETECTION OF MELANOMA

BACKGROUND OF THE INVENTION

Cutaneous malignant melanoma is a common, aggressive cancer with growing incidence. It is a serious healthcare problem with over 55,100 new cases anticipated in 2004 in US, and a mortality rate of about 14.5%. Cancer Facts and Figures 2003. American Cancer Society, 2003. The incidence of melanoma continues to rise faster than that of any other malignancy. De Braud et al. (2003). While prognosis of early local melanoma is favorable with 5-year overall survival over 90%, regional lymph node involvement decreases the overall survival rate to 10-46%. Balch et al. (2001). Therefore regional lymph node (LN) status becomes the most significant prognostic factor in a melanoma patient's survival. Introduction of the sentinel lymph nodes (SLN) technique (Morton (1992)) has increased the sensitivity of melanoma micrometastasis detection compared to H&E staining alone. Yu et al. (1999); and Messina et al. (1999). Nevertheless, even when enhanced by IHC, histological analysis is limited by the ability of light microscopy to recognize the tumor cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis has recently been proposed for a more sensitive detection of melanoma cells in LN. Many studies, when using well-characterized melanocyte specific markers, such as tyrosinase and MART-I, h ɔ /e demonstrated the presence of these gene transcripts in LNs oi.ierwise found to be negative by routine histology and IHC. Shivers et al. (1998); and Kuo et al. (2003). However, these genes are not specific to tumor cells and cannot be used to discriminate between benign and malignant tissue. hi fact, they caused false-positive results in the presence of benign capsular nevi. Takeuchi et al. (2004); Starz et al. (2003); and Gutzmer et al. (2002). Considering that benign nevi are not rare events in the melanoma SLN, the current RT-PCR assays are not useful clinically for diagnostic of melanoma micrometastasis. A recent study, proposed a multi-marker panel, including cancer specific markers for RT-PCR assay in order to increase assay specificity. Hoon et al. (2004). Identificatipn of novel melanoma specific markers remains one of the key questions of melanoma research.

Certain proteins have been shown to be associated with melanoma and its metastases. These proteins or their activities have been used in IHC to identify

metastases and include LlCAM (Thies et al. (2002); Fogel et al. (2003)); and S-100 (Diego et al. (2003)).

Nucleic acid tests have been proposed to increase the sensitivity of detection of metastatic melanoma. US Patent Publication Nos. 2002/01 10820; and 2003/0232356. Studies have used markers that include MAGE3 , tyrosinase, MART- 1, MITF-M or IL-I, Rl, endothelin-2, ephrin-A5, IGF Binding protein 7, HLA-A0202 heavy chain, Activin A (βA subunit), TNF RII, SPC4, CNTF Ra, or gplOO (HMB45) genes. Bostick et al. (1999); Hoon et al. (2001); Palmieri et al. (2001); Wrightson et al. (2001); Gutzmer et al. (2002); Davids et al. (2003); Starz et al. (2003); Rimboldi et al. (2003); Cook et al (2003); Reintgen et al. (2004); US Patent Publication Nos. 2002/0098535; 2003/0049701; US Patent Nos. 5,512,437; 5,512,444; 5,612,201; 5,759,783; 5,844,075; 6,025,474; 6,057,105; 6,235,525; 6,291,430; 6,338,947; 6,369,21 1; 6,426,217; 6,475,727; 6,500,919; 6,527,560; 6,599,699; WO 96/29430. Where determined, these markers have not been found adequate for sole use in melanoma diagnosis. Riccioni et al. (2002); Gutzmer et al. (2002); Davids et al. (2003); Goydos et al. (2003); and Prichard et al. (2003).

A number of these markers have also been shown to be indicative of other neoplasias such as ME20M (GPlOO) for clear cell sarcoma, biliary tract carcinoma and gastric carcinoma. Hiraga et al. (1997); Okada et al. (2001); Okami et al. (2001); Antonescu et al. (2002); Segal et al. (2003). MAGE3 is also indicative of a number of neoplasias including breast, hepatocellular, renal, neural, lung and esophageal. Yamanaka et al. (1999); Ooka et al. (2000); Suzuki et al. (2000); Cheung et al. (2001); and Weiser et al. (2001). Several melanoma antigen-encoding genes are also expressed in lung cancer. Yoshimatsu et al. (1998).

These markers proved to be sensitive but non-specific since they showed positive expression in other cancers and benign melanocytes. Additionally, tyrosinase is expressed in Schwann cells which are present in normal lymph nodes. The lack of specificity alone calls for the development of assays with new or additional markers. H&E histology and IHC remain the "gold standard for the identification of melanoma and nevus cells in SLNs." Starz et al. (2003). Detection issues in the intra-operative setting make this need even more acute.

Lymph node involvement is the strongest prognostic factor in many solid tumors, and detection of lymph node micrometastases is of great interest to pathologists and surgeons. Current lymph node evaluation involves microscopic examination of H&E-stained tissue sections and IHC and suffers from three major limitations: (a) small foci of cells, are easily missed; (b) the result is not rapidly available, meaning that any positive result in a SLN procedure requires a second surgery for removal of axcillary lymph nodes; and (c) only one or two tissue sections are studied, and thus the vast majority of each node is left unexamined. Serial sectioning can help overcome sampling error, and IHC can help identify small foci of cells; this combination, however, is costly and time consuming for routine analysis.

Surgical decisions of regional lymph node dissection can be based on intra-operative frozen section analysis of lymph nodes; however, the sensitivity of these methods is relatively poor, ranging from 50-70% relative to standard H&E pathology, leading to a high rate of second surgeries. Thus, pathologists are not routinely performing intra-operative frozen section analysis or touch print cytology analysis for melanoma patients. Improvements in the sensitivity and specificity of intra-operative assays for melanoma would significantly benefit oncology.

High-density microarrays have been applied to simultaneously monitor expression, in biological samples, of thousands of genes. Studies have resulted in the identification of genes differentially expressed in benign and malignant lesions, as well as genes that might be of prognostic value. Luo et al. (2001); and Wang et al. (2004). Gene expression profiling of malignant melanoma has been accomplished using a microarray containing probes for 8,150 cDNAs. Bittner et al. (2000). These researchers identified several genes that might be associated with aggressive tumor behavior. In recent work, comparison of gene expression profiles of a few melanoma and normal melanocyte cell lines led to the identification of differentially expressed genes and pathways modulated in melanoma. Takeuchi et al. (2004).

SUMMARY OF THE INVENTION

Gene expression profiling of an extensive set of clinically relevant tissue samples is provided in the present invention. Total RNA from forty-five primary malignant melanomas, 18 benign skin nevi and 7 normal skin tissues were hybridized on an

Affymetrix HuI 33A microarray containing 22,000 probe sets. Differentially expressed genes in malignant melanoma as compared to benign tissue were identified. Pathway analysis of the differentially expressed genes revealed an over-representation of genes associated with neural tissue development and activation of amyloid processing signaling pathway. A one-step quantitative RT-PCR assay was used to test a combination of two melanoma specific genes, PLAB and LICAM in a panel of clinically relevant samples that included primary malignant melanoma, benign nevi, melanoma LN metastasis and melanoma-free lymph node samples.

The present invention provides a method of identifying a melanoma by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding mRNA corresponding to prostate differentiation factor (PLAB, MICl) (SEQ ID NO: 1) and L1 cell adhesion molecule (LICAM) (SEQ ID NO: 2); or PLAB, LICAM and neurotrophic tyrosine kinase receptor, type 3 (NTRK3) (SEQ ID NO: 3) where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample. The invention further provides a method of identifying a melanoma by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15; or SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15 and SEQ ID NOs: 16-18 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

The invention also provides a method of distinguishing a malignant melanocyte from a benign melanocyte by obtaining a tissue sample; and assaying and measuring the expression levels in the sample of genes encoding PLAB and LICAM; or PLAB, LICAM and NTRK3 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

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The invention further provides a method of determining patient treatment protocol by obtaining a tissue sample from the patient; and assaying and measuring the expression levels in the sample of genes encoding PLAB and LICAM; or PLAB, LICAM and NTRK3 where the gene expression levels above pre-determined cut-off levels are indicative of the presence of a melanoma in the sample.

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The invention further provides additional Marker and control genes, the expression of which aid in the claimed methods. These additional genes include up-regulated SEQ ID NOs: 29-467 and down-regulated SEQ ID NOs: 468-978.

The primary Marker can be PLAB and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 1. PLAB is also described by Paralkar et al. (1998) and represented by Accession No. AF003934. PLAB is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 4-9.

The secondary Marker can be LICAM and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 2. LICAM is also described by Haspel et al (2003); and US Patent No. 6,107,476 and is represented by Accession No. NM_000425. LICAM is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 10-15.

The invention further provides a kit for conducting an assay to determine the presence of melanoma in a cell sample comprising: nucleic acid amplification and detection reagents.

The invention further provides primer/probe sets for amplification and detection of PCR products obtained in the inventive methods. These sets include the following:

SEQ ID NO:4 (PLAB forward primer) ggcagaatcttcgtccgca

SEQ ID NO: 5(PLAB reverse primer) ggacagtggtccccgttg

SEQ ID NO.6 (PLAB probe) cccagctggagttgcacttgcggcc

SEQ ID NO:7 (PLAB upper primer) gaacaccgacctcgtccc

SEQ ID NO: 8 (PLAB lower primer) ggcggcccgagagata

SEQ ID NO: 9 (PLAB probe) cgccagaagtgcggctgggattt

SEQ ID NO: 10 (LlCAM forward) gctgggactgggaacagaact

SEQ ID NO:11 (L lCAM Reverse) ggagcagagatggcaaagaaa

SEQ ID NO:12 (L lCAM probe) ttccccaccatctgctgt

SEQ ID NO: 13 (LlCAM upper) ccacagatgacatcagcctcaa

SEQ ID NO: 14 (LlCAM lower) ggtcacacccagctcttcctt

SEQ ID NO: 15 (LlCAM probe) tggcaagcccgaagtgcagttcctt

SEQ ID NO:16 (NTRK3 primer) gccccggcacccttta

SEQ ID NO:17 (NTRK3 primer) aaccctgccagtggtggat

SEQ ID NO: 18 (NTRK3 probe) cagatgggtgttttc

SEQ ID NO: 19 (Tyr upper) actcagcccagcatcattcttc

SEQ ID NO:20 (Tyr lower) atggctgttgtactcctccaatc

SEQ ID NO:21 (Tyr probe) cttctcctcttggcagattgtctgtagctt

SEQ ID NO:22 (PBGD upper) ccacacacagcctactttccaa

SEQ ID NO:23 (PBGD lower) tacccacgcgaatcactctca

SEQ ID NO:24 (PBGD probe) aacggcaatgcggctgcaacggcggaatt

The invention further provides amplicons obtained by PCR methods utilized in the inventive methods. These amplicons include the following:

SEQ ID NO:25 (PLAB Amplicon)
gaacaccgacctcgtcccggcccctgcagtccggatactcacgccagaagtgcggctgggatccggcggccacctgca
cctgcgtatctctcgggccgcc

SEQ ID NO:26 (LlCAM Amplicon)
ccacagatgacatcagcctcaagtgtgaggccagtggcaagcccgaagtgcagttccgctggacgagggatggtgtcca
cttcaaacccaaggaagagctgggtgtgacc

SEQ ID NO:27 (tyrosinase Amplicon)

actcagcccagcatcattcttctcctcttggcagattgtctgtagccgattggaggagtacaacagccat

SEQ ID NO:28 (PBGD Amplicon)
ccacacacagcctactttccaagcggagccatgtctggtaacggcaatgcggctgcaacggcggaagaaaacagcccaa
agatgagagtgattcgcgtgggta

Other genes described herein include up-regulated Markers (SEQ ID NOs: 29-467), down-regulated Markers (SEQ ID NOs: 468-978), PBGD (SEQ ID NO: 979), MARTl (SEQ ID NO: 980), ME20M (GPlOO; SEQ ID NO: 981) and MAGE-3 (SEQ ID NO: 982) and various primers and probes (SEQ ID NOs: 983-101 1) used in detecting their expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Flowchart of data analysis.

Fig. 2. Hierarchical clustering on the 15,795 genes that have at least two "present" calls in all samples. Each column is a sample and each row is a gene. Red is up-regulation and green is down-regulation. Purple: melanoma samples; yellow: benign nevi; and blue: normal skin.

Fig. 3. Microarray expression (A) and real time RT-PCR validation data (B) of the selected genes. First fourteen samples from the left are the melanoma tissue samples (red); next seven are benign nevi samples (yellow) and last five are normal skin (blue). For microarray plots x-axis shows intensity values; for PCR plots, x —axis is $2^{\Delta CT}$, where ΔCt is Ct (Target Gene) - Ct PBGD.

Fig. 4. Amyloid processing pathway. Adopted from Ingenuity™ Pathway Analysis Software Application. Genes up-regulated in melanoma are red and down-regulated in melanoma are green. Each gene symbol is followed by the fold-change of expression level between melanoma and benign/normal samples.

Fig. 5. One-step quantitative RT-PCR assay of PLAB and LlCAM (A) and conventional melanoma markers, gplOO, tyrosinase (SEQ ID NO: 999) and MARTl (B). For each plot x-axis represents score for the new markers or the conventional markers. Median scores for each samples category are labeled. Two cut-off levels based on normal (green) and benign (red) samples are labeled on each plot.

DETAILED DESCRIPTION

The present invention provides methods of qualitatively and quantitatively identifying a melanoma; distinguishing a malignant melanocyte from a benign melanocyte; diagnosing melanocytic lesions with uncertain pathological features; and determining a melanoma patient treatment protocol. The methods further provide aids in patient prognosis, patient monitoring and drug development. The methods rely on assaying and measuring expression levels of various Marker genes encoding mRNAs provided herein where gene expression over a pre-determined cut-off level is indicative of the presence of a malignant melanocyte in the sample assayed.

Cutaneous melanoma is a common, aggressive cancer with growing incidence. Identification of melanoma-specific deregulated genes could provide molecular markers for LN staging assays and further insight on melanoma tumorigenesis. Total RNA isolated from 45 primary melanoma, 18 benign skin nevi, and 7 normal skin tissue specimens were analyzed on an Affymetrix U133A microarray containing 22,000 probe sets. Hierarchical clustering revealed a distinct separation of the melanoma samples from benign and normal specimens. Novel genes associated with malignant melanoma were identified. Differential gene expression of two melanoma specific genes, PLAB and LICAM, were tested by a one-step quantitative RT-PCR assay on primary malignant melanoma, benign nevi and normal skin samples and also on malignant melanoma LN metastasis and melanoma-free lymph nodes. The performance of the markers was compared to conventional melanoma markers such as tyrosinase, gp100, and MART1 . The results demonstrated the ability of using a combination of PLAB and LICAM in a RT-PCR assay to differentiate clinically relevant tissue samples containing benign or malignant melanocytes.

High-density cDNA and oligonucleotide microarrays allow simultaneous monitoring of the expression of thousands of genes. Microarray technology provides a quantitative measurement of mRNA abundance and has gained acceptance as a tool for marker discovery based on gene expression. In the context of cancer research, microarray analysis has identified genes differentially expressed in benign and malignant lesions, different cancer types or that have prognostic significance. Luo et al. (2001); Su et al. (2001); Henshall et al. (2003); and Wang et al. (2004). The first

gene expression profiling of malignant melanoma used a microarray containing probes for 8,150 cDNAs and identified genes that might be associated with aggressive tumor behavior. Bittner et al. (2000). Since the samples analyzed in their study did not include tissues containing normal or benign melanocytes, differentially expressed genes in malignant melanoma were not identif ed. hi contrast to normal skin, melanocyte content in benign nevi is close to that in melanoma.

In another study, two pooled samples derived from either melanoma or benign nevi tissues were hybridized to a cDNA array and genes preferentially expressed in melanoma- or nevi-derived samples were found. Seykora et al. (2003). Other researchers used subtractive hybridization or analysis of SAGE libraries generated on melanoma cell lines, for monitoring gene expression in melanoma. Hipfel et al. (2000); and Weeraratna (2004). Recently, comparison of gene expression profiles of a few melanoma and melanocyte cell lines led to the identification of differentially expressed genes and pathways modulated in melanoma. Hoek et al. (2004). While these studies provide a solid foundation for melanoma genetics, there is no marker that can clearly differentiate melanoma from benign tissue. Several markers currently used such as tyrosinase and Mart-1 cannot discriminate between benign and malignant tissue. Takeuchi et al. (2004). Consequently, these markers have limited use in applications such as intra-operative, lymph-node-based staging of disease.

Difficulties in obtaining sufficient RNA samples from malignant melanoma and benign melanocyte lesions, tissue heterogeneity, and the presence of melanin in purified RNA remain the major challenges in these studies. In the study presented herein, total RNA isolated from 45 primary malignant melanomas, 18 benign skin nevi, and 7 normal skin tissues were hybridized on an Affymetrix HuI 33A microarray containing 22,000 probe sets. A modified RNA extraction method was developed to produce melanin-free RNA samples that increased the micorarray hybridization signals. Hierarchical clustering revealed distinct separation of the melanoma samples from benign and normal specimens. Significance Analysis of Microarray (SAM) method, *t-test* and percentile analysis identified 439 up-regulated (SEQ ID NOs: 29-467) and 511 down-regulated (SEQ ID NOs: 468-978) genes in the melanoma samples. Besides well-characterized genes such as me20m (gplOO), melanocortin

receptor 1, and L1CAM, many novel genes previously unassociated with melanoma were identified including NTKR3 and PLAB. Pathway analysis of the differentially expressed genes revealed an over-representation of genes associated with neural tissue development and function, activation of amyloid processing and integrin signalling pathways. RT-PCR assays were performed to confirm the differential expression of the selected genes.

The methods provided have sufficient specificity and sensitivity to detect metastasis of melanoma. A comparison of the current methods available indicates that tradition methods of H&E and IHC are clinically acceptable whereas, prior to the current invention, PCR methods were unacceptable. Table 1 shows the drawbacks and advantages of current methods prior to the invention claimed herein.

Table 1

Method	Sensitivity	Specificity
H&E	Low	100%
IHC	Low	100%
PCR	High	Low

In the present invention, specificity is preferably at least 95%, more preferably, specificity is at least 97% and most preferably, specificity is at least 99% based on a comparison of H&E and IHC negative nodes. Preferably, sensitivity is at least at least 80%, more preferably sensitivity is at least 85% and most preferably, sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes. Preferably, specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

Preferably, the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

The preferred methods of the invention employ a rapid technique for extracting nucleic acids from a tissue sample and a method of amplifying and detecting nucleic acid fragments indicative of metastasis. The nucleic acid fragments qualitatively and quantitatively measure mRNA encoded by the Marker genes. Tissue samples include lymph node, both regional and sentinel, skin lesions and other biopsy material.

The methods provided herein allow for intra-operative detection of micrometastases allowing a physician to determine whether to excise additional lymph nodes and to immediately implement an appropriate treatment protocol. As shown in Table 2, if a LN is found to be positive for melanoma, regional LNs are excised and interferon therapy could be suggested. Standard biopsy methods can take over one week and a positive result requires additional surgery to remove LNs and there is a concomitant delay in interferon therapy.

Table 2

Clinical Stage	1° Tumor (T)	LN	Metastasis	Treatment
Stage I	T1: ≤ 1 mm	Negative	Absent	Excision 1 cm
	T2: 1.01-2.00 mm	Negative	Absent	Excision 1-2 cm
Stage II	T3: 2.01-4.00 mm	Negative	Absent	Excision 2 cm
	T4: > 4.01 mm	Negative	Absent	Excision 2 cm
Stage III	Any thickness	Positive	Absent	Excision + complete LN dissection + interferon clinical trial
Stage IV	Any thickness	Positive	Present	Interferon clinical trial, symptomatic therapy

It is important to adequately sample the tissue used to conduct the assay. This includes proper excision and processing of the tissue sample as well as extraction of RNA. Once obtained, it is important to process the tissue samples properly so that any cancerous cells present are detected.

In the most preferred embodiment of the invention, node sampling is also given attention both intra- and extra-operatively. Since the distribution of cancer cells in nodes is non-uniform, it is preferable that multiple sections of the node be sampled. Every identified SLN should be submitted for pathological evaluation. SLN material is ordinarily be fixed in formalin and examined as formalin fixed, paraffin embedded tissue sample. Equally representative parts of SLN are processed for molecular analysis (fresh tissue) and histology (fixed tissue). General LN sampling procedures are described in Cochran et al. (2001); and Cochran et al. (2004). One method for accomplishing both a molecular based test and an examination of the same node sample by pathology is to bisect the node through the longest diameter. Each half is then divided into at least four full-faced sections with at least one outer and inner

section for pathology as fixed material, and at least one outer and inner section for molecular testing. As the distribution of metastases and micrometastases is not uniform in nodes or other tissues, a sufficiently large sample should be obtained so that metastases will not be missed. One approach to this sampling issue in the present method is to homogenize a large tissue sample, and subsequently perform a dilution of the well-mixed homogenized sample to be used in subsequent molecular testing.

In the case of LN tissue samples, it is preferable to remove any adipose tissue prior to cellular disruption. Manual cell and tissue disruption can be by any means known in the art such as a disposable tissue grinder described in US Patent 4,715,545 or a commercial homogenizer such as Omni GLHl 15 with disposable probes (Omni International, Warrenton, VA). Homogenization time is within 1 to 2 minutes and is more preferably 30-45 sec. The sample can then be processed to purify the RNA prior to assaying and measuring Marker expression levels. Suitable RNA purification methods include columns such as (e.g., RNeasy mini column, QIAshredder, QIAGEN Inc., Valencia, CA, or a suitable substitute).

A variety of techniques are available for extracting nucleic acids from tissue samples. Typical commercially available nucleic acid extraction kits take at least 15 minutes to extract the nucleic acid. In the preferred intra-operative methods of the instant invention, nucleic acid is extracted in less than 8 minutes and preferably less than 6 minutes.

The successful isolation of intact RNA generally involves four steps: effective disruption of cells or tissue, denaturation of nucleoprotein complexes, inactivation of endogenous ribonuclease (RNase) and removal of contaminating DNA and protein. The disruptive and protective properties of guanidinium isothiocyanate (GITC) and β-mercaptoethanol (β-me) to inactivate the ribonucleases present in cell extracts make them preferred reagents for the first step. When used in conjunction with a surfactant such as sodium dodecylsulfate (SDS), disruption of nucleoprotein complexes is achieved allowing the RNA to be released into solution and isolated free of protein. Tissues are homogenized in the GITC-containing lysis buffer, addition of ethanol creates the appropriate conditions for RNA to bind to the silica membrane. Centrifugation can clear the lysate of precipitated proteins and cellular DNA and is

preferably performed through a column. RNA purification is preferably conducted on a spin column containing silica or other material.

RNA is precipitated via the spin column as described above and centrifugation times are preferably no greater than 30 sec. Typically, the sample is diluted with an equal volume of 70% ethanol and thoroughly mixed prior to applying to the column. After washing, the column is dried by centrifugation, and RNA is eluted in RNase free water and collected by centrifugation. The total time of this rapid protocol is less than 8 minutes and preferably less than 6 min.

In summary the rapid RNA extraction method involves the following steps:

obtaining a tissue sample;

homogenizing the tissue to produce a homogenate;

contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;

allowing the RNA to bind to the RNA binding material;

washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and

eluting bound RNA from the substrate.

The reagents involved in this rapid extraction process can be those provided by the manufacturer or can be, for instance:

Lysis/Binding buffer (preferably, 4.5M GITC, 10OmM $NaPO_4$),

Wash buffer I (preferably, 37% ethanol in 5M GITC, 2OmM Tris-HCl),

Wash buffer II (preferably, 80% ethanol in 2OmM NaCl, 2mM Tris-HCl), and

Nuclease-free sterile double distilled water for elution.

In one method, prior to the process for isolating nucleic acids described above, tissue samples are weighed and put into 8 or 14 ml polypropylene culture tubes and pre-cooled on dry ice. The frozen tissue samples are then divided into pieces of about 50 mg or less without being thawed. All buffers are those provided by QIAGEN in the RNeasy mini kit. A volume of homogenization (lysis) buffer is added to the tissue based on Table 3.

Table 3

Tissue Weight (mg)	Homogenization buffer (ml)
≤ 100	2
100-149	2
150-199	3
200-249	4
250-299	5
300-349	6
350-399	7
400-449	8
450-400	9
500-550	10
>550	*

* Tissue above 550 mg is divided into equivalent parts and processed as individual samples. An alternative method to calculate lysis buffer volume for tissues over 100 mg is to add 1 ml per 50mg tissue; using 2 ml for tissues less than 100 mg.

The tissue sample is then homogenized for instance by the Omni GLHl 15 at a power setting to grade 6, Adaptor AlOOO and disposable probes. The homogenate is then mixed with an equal volume of 70% ethanol and thoroughly mixed for instance by vortexing on a VWR Model G560 set at 10 speed (maximum) about 10 seconds or by pipetting 4-5 times. The homogenate/ethanol mixture is then applied to an RNeasy mini column mounted on a vacuum manifold in a volume in accordance with Table 4 so that a consistent amount of the original tissue (approximately 5 mg/column) is loaded thus producing comparable RNA yields for each tissue sample.

Table 4

Tissue weight (mg)	Volume homogenate/ethanol mix (µl) (recommended)
30-39	700
40-49	500
50-59	400
60-69	350
70-79	300
80-89	250
90-99	225
> 100	200

A vacuum is then applied to the column to remove the liquid. The vacuum is stopped and two washes of 700 ml are applied, first with RWI buffer and second with

RPE buffer each removed by filtration. Vacuum is at 800-1200 mBar in each case. The column is then placed into a 1.5 ml collection tube and centrifuged in an Eppendorf 5415D centrifuge at 13,200 rpm for 30 seconds to dry. The column is transferred to a new 1.5 ml collection tube. Fifty µl RNase-free water is directly added to the membrane and the column is centrifuged in an Eppendorf 541 5D centrifuge for 30 seconds at 13,200 rpm to elute the RNA. The RNA quality is determined with an Agilent Bioanalyzer and the RNA is stored at -70 ^{0}C.

Melanin can negatively impact the efficiency of reversed transcription and amplification reactions. Accordingly, a melanin removal process is undertaken when the sample is suspected of containing a significant amount of melanin (as in the case of samples of a primary melanoma or benign skin nevi) and is less of a concern when performing the assay on a SLN since melanocyte content is low. If necessary, melanin is removed to enhance reverse transcription and/or nucleic acid amplification.

Typically, melanin is removed during the filtration steps provided above. In the case of tissue with high melanin concentration, less tissue should be used, approximately 5 mg per Qiagen RNeasy mini column.

If another method is used that results in residual melanin in the sample, removal involves the use of a matrix employing a polymer bead system such as Bio-Gel P-60 (Bio-Rad Laboratories, Hercules, CA). Such a method is described by Satyamoorthy et al. (2002). Essentially, this method involves preparing a 50% (w/v) mixture of the Bio-Gel material in 10 mM sodium acetate (pH 4.2). About 300 µl of the mixture are placed in a micro-centrifuge tube and centrifuged at lOOOrpm for 1 min. The supernatant is discarded and the beads are placed in a mini-column or similar vessel. Homogenate is then passed through the vessel containing the beads (after first incubating them in the vessel). The supernatant is collected. Further washing of the beads with additional 100 µl aliquots of 10 mM sodium acetate can be used to capture additional volumes of melanin-free sample if necessary for adequate assay volume. The dark melanin will be clearly visible on the beads retained in the vessel. Other silica-based filters can also be used to remove the melanin pigment as described by Wang et al. (2001).

An important aspect of the intra-operative methods of the invention is rapid Marker detection. Provided that such methods can be conducted within a period acceptable for an intra-operative assay (i.e., no more than about 35 minutes), any reliable, sensitive, and specific method can be used.

In the case of measuring mRNA levels to determine gene expression, assays can be by any means known in the art and include methods such as PCR, Rolling Circle Amplification (RCA), Ligase Chain Reaction (LCR), Strand Displacement Amplification (SDA), Nucleic Acid Sequence Based Amplification (NASBA), and others. The rapid molecular diagnostics involved are most preferably quantitative PCR methods, including QRT-PCR. Detection can be by any method known in the art including microarrays, gene chips and fluorescence.

A typical PCR includes multiple amplification steps, or cycles that selectively amplify target nucleic acid species. A typical PCR includes three steps: a denaturing step in which a target nucleic acid is denatured; an annealing step in which a set of PCR primers (forward and backward primers) anneal to complementary DNA strands; and an elongation step in which a thermostable DNA polymerase elongates the primers. By repeating this step multiple times, a DNA fragment is amplified to produce an amplicon, corresponding to the target DNA sequence. Typical PCR includes 20 or more cycles of denaturation, annealing and elongation. Often, the annealing and elongation steps can be performed concurrently, in which case the cycle contains only two steps.

In the preferred inventive method, employing RT-PCR, the RT-PCR amplification reaction is conducted in a time suitable for intra-operative diagnosis, the lengths of each of these steps can be in the seconds range, rather than minutes. Specifically, with certain new thermal cyclers being capable of generating a thermal ramp rate of at least about 5C° per second, RT-PCR amplifications in 30 minutes or less are used. More preferably, amplifications are conducted in less than 25 minutes. With this in mind, the following times provided for each step of the PCR cycle do not include ramp times. The denaturation step may be conducted for times of 10 seconds or less. In fact, some thermal cyclers have settings for "0 seconds" which may be the optimal duration of the denaturation step. That is, it is enough that the thermal cycler reaches

the denaturation temperature. The annealing and elongation steps are most preferably less than 10 seconds each, and when conducted at the same temperature, the combination annealing/elongation step may be less than 10 seconds. Some homogeneous probe detection methods, may require a separate step for elongation to maximize rapid assay performance. In order to minimize both the total amplification time and the formation of non-specific side reactions, annealing temperatures are typically above 50^{0}C. More preferably annealing temperatures are above 55°C.

A single combined reaction for RT-PCR, with no experimenter intervention, is desirable for several reasons: (1) decreased risk of experimenter error; (2) decreased risk of target or product contamination; and (3) increased assay speed. The reaction can consist of either one or two polymerases. In the case of two polymerases, one of these enzymes is typically an RNA-based DNA polymerase (reverse transcriptase) and one is a thermostable DNA-based DNA polymerase. To maximize assay performance, it is preferable to employ a form of "hot start" technology for both of these enzymatic functions. US Patents 5,41 1,876 and 5,985,619 provide examples of different "hot start" approaches. Preferred methods include the use of one or more thermoactivation methods that sequester one or more of the components required for efficient DNA polymerization. US Patents 5,550,044 and 5,413,924 describe methods for preparing reagents for use in such methods. US Patent 6,403,341 describes a sequestering approach that involves chemical alteration of one of the PCR reagent components. In the most preferred embodiment, both RNA- and DNA-dependent polymerase activities reside in a single enzyme. Other components that are required for efficient amplification include nucleoside triphosphates, divalent salts and buffer components. In some instances, non-specific nucleic acid and enzyme stabilizers may be beneficial.

In the preferred RT-PCR, the amounts of certain reverse transcriptase and the PCR components are atypical in order to take advantage of the faster ramp times of some thermal cyclers. Specifically, the primer concentrations are very high.

Typical gene-specific primer concentrations for reverse transcriptase reactions are less than about 20 nM. To achieve a rapid reverse transcriptase reaction on the order of one to two minutes, the reverse transcriptase primer concentration is raised to

greater than 20 nM, preferably at least about 50 nM, and typically about 100 nM. Standard PCR primer concentrations range from 100 nM to 300 nM. Higher concentrations may be used in standard PCR to compensate for Tm variations. However, for the purposes herein, the referenced primer concentrations are for circumstances where no Tm compensation is needed. Proportionately higher concentrations of primers may be empirically determined and used if Tm compensation is necessary or desired. To achieve rapid PCR, the PCR primer concentrations typically are greater than 250 nM, preferably greater than about 300 nM and typically about 500 nM.

Commercially used diagnostics also preferably employ one or more internal positive control that confirms the operation of a particular amplification reaction in case of a negative result. Potential causes of false negative results that must be controlled in an RT-PCR include: inadequate RNA quantity, degradation of RNA, inhibition of RT and/or PCR and experimenter error.

In the case of measuring protein levels to determine gene expression, any method known in the art is suitable provided it results in adequate specificity and sensitivity. For example, protein levels can be measured by binding to an antibody or antibody fragment specific for the protein and measuring the amount of antibody-bound protein. Antibodies can be labeled by radioactive, fluorescent or other detectable reagents to facilitate detection. Methods of detection include, without limitation, enzyme-linked immunosorbent assay (ELISA) and immunoblot techniques.

The invention provides specificity and sensitivity sufficient to identify a malignant melanocyte in a tissue sample. The methods determine expression of particularly Marker genes by measuring mRNA encoded by the Markers. The preferred Markers of the invention display at least a two-fold over-expression in tissue having malignant melanocytes relative to benign melanocyte or normal tissue. The results presented herein show that a primary Marker is insufficient to provide clinically relevant information but, when combined with one or more secondary Markers, the information obtained compares to the "gold standard" of H&E and IHC upon which clinicians currently rely. Tertiary Markers and control genes can augment the primary and secondary Markers to further increase specificity and/or sensitivity.

As described in the following Examples, the Markers were identified by the protocol depicted in Figure 1. Thus, the invention provides a method for identifying melanoma-specific Markers by following the protocol in Figure 1 and the Examples provided herein.

The primary Marker can be PLAB and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 1. PLAB is also described by Paralkar et al. (1998) and represented by Accession No. AF003934. PLAB is linked to the pathogenesis of prostate cancer (Liu et al (2003); Karan et al. (2003); and Nakamura et al. (2003); US Patent Nos. 5,994,102; 6,107,476; 6,465,181; 6,500,638; 6,521,227; US Patent Publication Nos. 2002/0048784; 2003/0013097; and 2003/0059431) and colorectal cancer (Brown et al. (2003); Buckhaults et al. (2001); and US Patent Publication No. 2002/0160382).

The secondary Marker is LlCAM and is defined herein as the gene encoding any variant, allele etc. including SEQ ID NO: 2. LlCAM is also described by Haspel et al (2003); and US Patent Nos. 5,872,225; and 5,969,124 and is represented by Accession No. NM_000425.

The invention further provides tertiary markers that fall into several functional categories. Thus, additional Markers can be used that are found in these functional categories. As described in more detail in the Examples, melanoma-specific up-regulated genes fall into the functional categories of neural tissue development and cell cycle control and melanoma-specific down-regulated genes fall into the functional categories of tissue development and cell differentiation.

The tertiary Markers include SEQ ID NOs: 3, 29-978 and 999. A number of tertiary markers are described in Table 5 and all are summarized in Table 15.

NTRK3 is described by Strausberg et al. (2002); Marchetti et al. (2003); Hisaoka et al. (2002); McGregor et al. (1999); Ryden et al. (1996); US Patent Nos. 5,348,856; 5,844,092; 5,910,574; and US Patent Publication Nos. 2002/0155480; and 2003/014283 and is represented by Accession No. BC013693 or S76476.1. NTRK3 is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 16-18.

Tyrosinase is described by Mandelcorn-Monson et al. (2003); and US Patent No. 6,153,388 and is represented by Accession No. NM_000372. Tyrosinase is also defined as the gene encoding mRNA recognized by the primer/probe sets SEQ ID NOs: 19-21.

Table 5

Gene	Reference	Accession #
PBGD	Raich et al. (1986)	NM_000190
CITED1	Fenner et al. (1998)	NM_004143
PEX6	Raas-Rothschild et al. (2002)	NM_000287
CAPG	Van Impe et al. (2003)	NM_001747
DUSP4	Smith et al. (1997)	NM_001394
GDF1	Ducy et al. (2000)	NM_001492
E2-EpF	Liu et al. (1992)	NM_014501
me20m	Maresh et al. (1994)	U01874
CDH3	Patel et al. (2003)	NM_001793
SMARCD3	Ring et al. (1998)	NM_003078
PKM2	Luftner et al. (2003)	NM_002654
GPI	Tsutsumi et al. (2003)	NM_000175
Pig10	Polyak et al. (1997)	AF010413
CPEB1	Welk et al. (2001)	NM_030594
HOXHB9	Catala et al. (2002)	AI738662
Truncated calcium binding protein	The Washington University-Merck EST Project Hillier et al. (1995)	N30649
SAAS	Kikuchi et al. (2003)	NM_013271
HS1-2	Edgar et al. (2002)	NM_007011
HCN2	Stieber et al. (2003)	NM_001194
MBP	Kamholz et al. (1996)	M13577
AD3LPAD5	Li et al. (1995)	U34349
PLOD3	Wang et al. (2002)	NM_001084
MC1R	Salazar-Onfray et al. (2002)	
MIF	Shimizu et al. (1999)	NM_002415
HOXB7	Care et al. (1996)	NM_004502
AIM1	Ray et al. (1997)	XM_166300
EpHB6	Hafner et al. (2003)	NM_004445
AKT1	Majumder et al. (2004)	NM_005163
AKT2	Gosmanov et al. (2004)	NM_001626
AKT3	Xu et al. (2003)	NM_005465
APH-1A	Xu et al. (2003)	NM_016022
APP	Masters et al. (1985)	NM_201414
BACE	Pastorino et al. (2004)	NM_138973
BACE2	Pastorino et al. (2004)	NM_012104
CAPN1	Altznauer et al. (2004)	NM_005186
CAPN2	Alexa et al. (2004)	NM_001748

CDK5	Qi et al. (2004)	NM_004935
CDK5R1	Kam et al. (2004)	NM_003885
CSNK1A1	Burzio et al. (2002)	NM_001892
CSNK1D	Li et al. (2004)	NM_139062
CSNK1E	Swiatek et al. (2004)	NM_152221
CSNK2A1	Hilgard et al. (2004)	NM_001895
CSNK2A2	Szebeni et al. (2003)	NM_001896
CSN2K2B	Lee et al. (2004)	NM_001320
GSK3B	Chen et al. (2003)	NM_182946
MAPK1	Nishihara et al. (2004)	NM_138957
MAPK14	Bendotti et al. (2004)	NM_139014
MAPK3	Nishihara et al. (2004)	NM_002746
MAPT	Yu et al. (2004)	NM_016841
NCSTN	Shirotani et al. (2004)	NM_015331
PEN2	Marlow et al. (2003)	NM_172341
PRKACA	Sakwe et al. (2004)	NM_207518
PRKACB	Dwivedi et al. (2004)	NM_002731
PRKACG	Zhang et al. (2004)	NM_002732
PRKAR1A	Gronholm et al. (2003)	NM_212472
PRKAR2A	MacDougall et al. (2003)	NM_004157
PRKAR2B	Dwivedi et al. (2004)	NM_002736
PRKCE	Schechtman et al. (2004)	NM_005400
PSEN1	Pitsi et al. (2004)	NM_000021
PSEN2	Zatti et al. (2004)	NM_012486
PSFL	Clark et al. (2003)	NM_031301
ABL1	Gustafson et al. (2004)	NM_007313
ACK1	Ahmed et al. (2004)	NM_005781
ACTN4	Menez et al. (2004)	NM_004924
ARF1	Kadaja et al. (2004)	NM_001658
ARPC1B	Kaneda et al. (2002)	
BCAR3	Clark et al. (2003)	NM_003567
BRAF	Sasaki et al. (2004)	NM_004333
CDC42	Chen et al. (2004)	NM_044472
CRK	Stoletov et al. (2004)	NM_016823
CRKL	Zhang et al. (2003)	NM_005207
DDEF1	Oda et al. (2003)	NM_018482
DOCK1	Grimsley et al. (2004)	NM_001380
FYN	Lee et al. (2004)	NM_153048
GIT1	Haendeler et al. (2003)	NM_014030
GRB2	Zhou et al. (2004)	NM_203506
GRF2	Arozarena et al. (2004)	NM_006909
HRAS	Nomura et al. (2004)	NM_005343
JUN	Schmuth et al. (2004)	NM_002228
KRAS2	Qi et al. (2004)	NM_033360
MAP2K1	Rhee et al. (2004)	NM_002755

MAP2K2	Chen et al. (2004)	NM_030662
MAP2K4	Woo et al. (2004)	NM_003010
MAP3K11	Zhang et al. (2004a)	NM_002419
MAPK8	Fujii et al. (2004)	NM_139049
MYLK	Oury et al. (2004)	NM_053032
NRAS	Reifenberger et al. (2004)	NM_002524
PAK1	Sells et al. (1997)	HSU24152
PAK2	Kirchhoff et al. (2004)	NM_002577
PAK3	Kitano et al. (2003)	NM_002578
PAK4	Barac et al. (2004)	NM_005884
PAK6	Ching et al. (2003)	NM_020168
PAK7	Jaffer et al. (2002)	NM_020341
PTK2	Golubovskaya et al. (2004)	NM_005607
PXN	Saito et al. (2004)	NM_002859
RAC1	Pontow et al. (2004)	NM_198829
RAF1	Akula et al. (2004)	NM_002880
RAP1A	Nomura et al. (2004)	NM_002884
RAP2B	Evellin et al. (2002)	NM_002886
SHC1	Yannoni et al. (2004)	NM_183001
SOS1	Buchs et al. (2004)	NM_005633
SRC	Encinas et al. (2004)	NM_198291
TLN1	Tremuth et al. (2004)	NM_006289
VASP	Tokuo et al. (2004)	NM_003370
VCL	Izard et al. (2004)	NM_003373
WASPIP	Luthi et al. (2003)	NM_003387
ZYX	Li et al. (2004)	NM_003461

Tertiary Markers are able to replace and/or supplement primary or secondary Markers provided that the resulting assays have adequate sensitivity and specificity.

The specificity of any given amplification-based molecular diagnostic relies heavily, but not exclusively, on the identity of the primer sets. The primer sets are pairs of forward and reverse oligonucleotide primers that anneal to a target DNA sequence to permit amplification of the target sequence, thereby producing a target sequence-specific amplicon. The primers must be capable of amplifying Markers of the disease state of interest. In the case of the instant invention, these Markers are directed to melanoma.

The reaction must also contain some means of detection of a specific signal. This is preferably accomplished through the use of a reagent that detects a region of DNA sequence derived from polymerization of the target sequence of interest. Preferred reagents for detection give a measurable signal differential when bound to a specific

nucleic acid sequence of interest. Often, these methods involve nucleic acid probes that give increased fluorescence when bound to the sequence of interest. Typically, the progress of the reactions of the inventive methods are monitored by analyzing the relative rates of amplicon production for each PCR primer set.

The invention further includes primer/probe sets and their use in the claimed methods. The sequences are:

SEQ ID NO:4 (PLAB forward primer) ggcagaatcttcgtccgca

SEQ ID NO: 5(PLAB reverse primer) ggacagtggtccccgttg

SEQ ID NO: 6 (PLAB probe) cccagctggagttgcacttgcggcc

SEQ ID NO: 7 (PLAB upper primer) gaacaccgacctcgtccc

SEQ ID NO: 8 (PLAB lower primer) ggcggcccgagagata

SEQ ID NO: 9 (PLAB probe) cgccagaagtgcggctgggattt

SEQ ID NO: 10 (LlCAM forward) gctgggactgggaacagaact

SEQ ID NO:1 1 (LlCAM Reverse) ggagcagagatggcaaagaaa

SEQ ID NO:12 (LlCAM probe) ttccccaccatctgctgt

SEQ ID NO: 13 (LlCAM upper) ccacagatgacatcagcctcaa

SEQ ID NO:14 (LlCAM lower) ggtcacacccagctcttcctt

SEQ ID NO: 15 (LlCAM probe) tggcaagcccgaagtgcagttcctt

SEQ ID NO: 16 (NTRK3 primer) gccccggcacccttta

SEQ ID NO:17 (NTRK3 primer) aaccctgccagtggtggat

SEQ ID NO: 18 (NTRK3 probe) cagatgggtgttttc

SEQ ID NO: 19 (Tyr upper) actcagcccagcatcattcttc

SEQ ID NO:20 (Tyr lower) atggctgttgtactcctccaatc

SEQ ID NO:21 (Tyr probe) cttctcctcttggcagattgtctgtagctt

SEQ ID NO:22 (PBGD upper) ccacacacagcctactttccaa

SEQ ID NO:23 (PBGD lower) tacccacgcgaatcactctca

SEQ ID NO:24 (PBGD probe) aacggcaatgcggctgcaacggcggaatt

Monitoring amplicon production may be achieved by a number of detection reagents and methods, including without limitation, fluorescent primers, and fluorogenic probes and fluorescent dyes that bind double-stranded DNA. Molecular beacons, Scorpions, and other detection schemes may also be used. A common

method of monitoring a PCR employs a fluorescent hydrolysis probe assay. This method exploits the 5' nuclease activity of certain thermostable DNA polymerases (such as Taq or Tfl DNA polymerases) to cleave an oligomeric probe during the PCR process.

The invention further provides amplicons obtained by PCR methods utilized in the inventive methods. These amplicons include the following:

SEQ ID NO:25 (PLAB Amplicon)

gaacaccgacctcgtcccggcccctgcagtccggatactcacgccagaagtgcggctgggatccggcggccacctgca cctgcgtatctctcgggccgcc

SEQ ID NO:26 (Ll CAM Amplicon)

ccacagatgacatcagcctcaagtgtgaggccagtggcaagcccgaagtgcagttccgctggacgagggatggtgtcca cttcaaacccaaggaagagctgggtgtgacc

SEQ ID NO:27 (tyrosinase Amplicon)

actcagcccagcatcattcttctcctcttggcagattgtctgtagccgattggaggagtacaacagccat

SEQ ID NO:28 (PBGD Amplicon)

ccacacacagcctactttccaagcggagccatgtctggtaacggcaatgcggctgcaacggcggaagaaaacagcccaa agatgagagtgattcgcgtgggta

The oligomer is selected to anneal to the amplified target sequence under elongation conditions. The probe typically has a fluorescent reporter on its 5' end and a fluorescent quencher of the reporter at the 3' end. So long as the oligomer is intact, the fluorescent signal from the reporter is quenched. However, when the oligomer is digested during the elongation process, the fluorescent reporter is no longer in proximity to the quencher. The relative accumulation of free fluorescent reporter for a given amplicon may be compared to the accumulation of the same amplicons for a control sample and/or to that of a control gene, such as, without limitation, β-Actin or PBGD to determine the relative abundance of a given cDNA product of a given RNA in a RNA population. Products and reagents for the fluorescent hydrolysis probe assay are readily available commercially, for instance from Applied Biosystems.

Suitable detection reagents are commonly referred to as "Scorpions" and are described in US Patents 6,326,145 and 5,525,494. These reagents include one or more molecules comprising a tailed primer and an integrated signaling system. The

primer has a template binding region and a tail comprising a linker and a target binding region. The target binding region in the tail hybridizes to complementary sequence in an extension product of the primer. This target specific hybridization event is coupled to a signaling system wherein hybridization leads to a detectable change. In PCR the target binding region and the tail region are advantageously arranged such that the tail region remains single stranded, i.e. uncopied. Thus the tail region is non-amplifiable in the PCR amplification products. The linker comprises a blocking moiety that prevents polymerase mediated chain extension on the primer template.

The most preferred detection reagents are TaqMan® probes (Roche Diagnostics, Branchburg, NJ) and they are described in US Patents 5,210,015; 5,487,972; and 5,804,375. Essentially, these probes involve nucleic acid detection by virtue of the separation of a fluor-quencher combination on a probe through the 5'-3' exonuclease activity of the polymerase used in the PCR. Any suitable fluorophore can be used for any of the Markers or controls. Such fluorophores include, without limitation, Texas Red, CaI Red, Fam, Cy3 and Cy5. In one embodiment, the following fluorophores correspond to the noted Markers: PLAB: Fam; LlCAM: Texas Red or CaI Red, tyrosinase: Cl; PBGD: Cy5.

Equipment and software also are readily available for controlling and monitoring amplicon accumulation in PCR and QRT-PCR including the Smart Cycler thermocylcer commercially available from Cepheid of Sunnyvale, California, and the ABI Prism 7700 Sequence Detection System, commercially available from Applied Biosystems.

In the case of gene expression assays, it is preferable to use a gene constitutively expressed in the tissue of interest. PBGD is commonly used as an internal control due to several factors: it contains no known pseudogenes in humans, it is constitutively expressed in human tissues and it is expressed at a relatively low level and therefore is less likely to cause inhibition of the amplification of target sequences of interest. Use of PBGD as a control minimizes or eliminates reporting erroneous results arising from all potential sources of false negative results.

In the commercialization of the described methods for QRT-PCR certain kits for detection of specific nucleic acids are particularly useful. In one embodiment, the kit includes reagents for amplifying and detecting Markers. Optionally, the kit includes sample preparation reagents and or articles (e.g., tubes) to extract nucleic acids from lymph node tissue. The kits may also include articles to minimize the risk of sample contamination (e.g., disposable scalpel and surface for lymph node dissection and preparation).

In a preferred kit, reagents necessary for the one-tube QRT-PCR process described above are included such as reverse transcriptase, a reverse transcriptase primer, a corresponding PCR primer set (preferably for Markers and controls), a thermostable DNA polymerase, such as Taq polymerase, and a suitable detection reagent(s), such as, without limitation, a scorpion probe, a probe for a fluorescent hydrolysis probe assay, a molecular beacon probe, a single dye primer or a fluorescent dye specific to double-stranded DNA, such as ethidium bromide. The primers are preferably in quantities that yield the high concentrations described above. Thermostable DNA polymerases are commonly and commercially available from a variety of manufacturers. Additional materials in the kit may include: suitable reaction tubes or vials, a barrier composition, typically a wax bead, optionally including magnesium; reaction mixtures (typically 10X) for the reverse transcriptase and the PCR stages, including necessary buffers and reagents such as dNTPs; nuclease-or RNase-free water; RNase inhibitor; control nucleic acid(s) and/or any additional buffers, compounds, co-factors, ionic constituents, proteins and enzymes, polymers, and the like that may be used in reverse transcriptase and/or PCR stages of QRT-PCR. Optionally, the kits include nucleic acid extraction reagents and materials. Instructions are also preferably included in the kits.

The following examples are provided to illustrate but not limit the claimed invention. AU references cited herein are hereby incorporated herein by reference.

Example 1
Tissue Preparation

Fresh frozen malignant melanoma, benign skin nevi, normal skin, melanoma lymph node metastasis and melanoma-free lymph node samples were obtained from Genomics Collaborative, Inc. (Cambridge, MA), Asterand (Detroit, MI), Clinomics (Pittsfield, MA) and Proteogenex (Los Angeles, CA), Ardais (Lexington, MA) and Impath (Westborough, MA). All tissue vendors declared that tissue specimens used in the study were collected according to an Institutional Review Board approved protocol of corresponding hospitals and principles of bioethics. Patients' demographic and pathology information was also collected. The histopathological features of each sample were reviewed to confirm diagnosis, and to estimate sample preservation and tumor content.

Melanoma and benign nevi primary tissues chosen for microarray analysis had melanocyte content greater than 50% with no mixed histology. Melanoma positive lymph nodes were collected from malignant melanoma patients; diagnosis of melanoma was confirmed by H&E in combination with IHC (SlOO and HMB45). Melanoma free lymph nodes derived from patients that did not have melanoma in their clinical history and absence of melanoma was confirmed by H&E and IHC using antibodies for SlOO and HMB45.

RNA from a total of 70 primary tissue samples was used for gene expression profiling and melanoma specific gene identification. Samples included 45 primary malignant melanoma, 18 benign skin nevi, and 7 normal skin tissues. The majority of primary melanomas included in the study represent early stage of disease and have thickness less than 4 mm, which is consistent with the standard melanoma patient population. Aitken et al. (2004). Patient demographic, clinical and pathology characteristics are presented in Table 6 and summarized in Table 7.

In addition, 77 malignant melanoma LN metastasis and 18 melanoma-free LN tissue samples were used for one-step quantitative PCR assay. Melanoma positive lymph nodes included axillary, cervical and inguinal lymph nodes with metastasis derived from epithelioid and spindle cell primary melanomas. Out of 18 melanoma free LN, 10 were collected from other cancer patients but no cancer cells were found in these nodes by pathologists and 8 LN were from non-malignant lesions.

Table 6

Sample ID	Age	Gender	Race	Diagnosis	Location	T Stage	N&M Stage	Clark level
430MM	n/a	F	Cau	normal skin	trunk			
431MM	n/a	F	Cau	normal skin	trunk			
432MM	n/a	F	Cau	normal skin	trunk			
433MM	n/a	F	Cau	normal skin	trunk			
435MM	n/a	F	Cau	normal skin	trunk			
437MM	n/a	F	Cau	normal skin	trunk			
485MM	37	M	Cau	normal skin	skin, NOS			
487MM	35	F	Cau	atypical nevus, mild	face			
489MM	56	F	Cau	compound nevus	face			
490MM	16	F	Cau	compound nevus	scalp & neck			
491MM	15	M	Cau	compound nevus	trunk			
493MM	35	F	Cau	compound nevus	trunk			
495MM	18	F	Cau	benign nevus, NOS	trunk			
496MM	21	F	Cau	intradermal nevus	lower limb & hip			
497MM	12	M	Cau	intradermal nevus	lower limb & hip			
498MM	44	F	Cau	benign nevus, NOS	trunk			
499MM	48	F	Cau	benign nevus, NOS	face			
500MM	65	M	Cau	intradermal nevus	trunk			
501MM	30	M	Cau	compound nevus	lower limb & hip			
502MM	20	F	Cau	compound nevus	trunk			
503MM	35	M	Cau	intradermal nevus	lower limb & hip			
504MM	23	M	Cau	compound nevus	trunk			
507MM	53	M	Cau	atypical nevus, moderate	trunk			
508MM	28	M	Cau	compound nevus	trunk			
509MM	43	M	Cau	intradermal nevus	trunk			
392MM	58	F	Cau	epithelioid melanoma	trunk	T3	N0M0	4
397MM	51	F	Cau	epithelioid melanoma	lower limb & hip	T2	N0M0	3
405MM	46	M	Cau	epithelioid melanoma	upper limb & shoulder	T2	N0M0	3
407MM	64	F	Cau	epithelioid melanoma	trunk	T1	N0M0	2
409MM	54	F	Cau	epithelioid melanoma	scalp & neck	T2	N0M0	3
440MM	61	M	Cau	malignant melanoma, NOS	lower limb & hip	T1	N0M0	2
441MM	78	M	Cau	spindle cell melanoma	face	T4	N0M0	5
442MM	52	M	Cau	malignant melanoma, NOS	upper limb & shoulder	T2	N0M0	3
443MM	51	F	Cau	spindle cell melanoma	trunk	T2	N0M0	3
444MM	49	F	Cau	spindle cell melanoma	lower limb & hip	T3	N0M0	4
445MM	76	F	Cau	malignant melanoma, NOS	upper limb & shoulder	T3	N0M0	4
446MM	86	M	Cau	malignant melanoma, NOS	scalp & neck	T1	N0M0	2
447MM	48	M	Cau	epithelioid melanoma	skin, NOS	T3	N0M0	4
448MM	72	F	Cau	epithelioid melanoma	upper limb & shoulder	T2	N0M0	3
449MM	62	M	Asian	epithelioid melanoma	lower limb & hip	T3	N1M0	n/a
450MM	90	F	Cau	epithelioid melanoma	upper limb & shoulder	T4	N1M1	n/a
452MM	43	M	Cau	epithelioid melanoma	skin, NOS	T3	N0M0	n/a
453MM	48	F	Cau	epithelioid melanoma	trunk	T3	N0M0	n/a
454MM	69	M	Cau	epithelioid melanoma	upper limb & shoulder	T3	N0M0	n/a
455MM	55	M	Cau	malignant melanoma, NOS	skin, NOS	T2	N0M0	n/a
456MM	63	M	Cau	malignant melanoma, NOS	lower limb & hip	T2	N0M0	3

457MM	69	M	Cau	spindle cell melanoma	trunk	T1	N0M0	2
459MM	86	F	Cau	malignant melanoma, NOS	lower limb & hip	T2	N0M0	3
460MM	64	M	Cau	malignant melanoma, NOS	upper limb & shoulder	T3	N0M0	4
461MM	66	M	Cau	epithelioid melanoma	trunk	T1	N0M0	2
463MM	58	M	Cau	malignant melanoma, NOS	trunk	T1	N0M0	2
464MM	53	M	Cau	epithelioid melanoma	face	T2	N0M0	3
465MM	77	F	Cau	epithelioid melanoma	upper limb & shoulder	T3	N0M0	4
466MM	79	F	Cau	malignant melanoma, NOS	upper limb & shoulder	T1	N0M0	2
468MM	86	F	Cau	spindle cell melanoma	upper limb & shoulder	T2	N0M0	3
469MM	43	F	Cau	malignant melanoma, NOS	scalp & neck	T1	N0M0	2
470MM	81	M	Cau	malignant melanoma, NOS	upper limb & shoulder	T2	N0M0	3
472MM	38	F	Cau	spindle cell melanoma	upper limb & shoulder	T1	N0M0	2
473MM	69	F	Cau	malignant melanoma, NOS	upper limb & shoulder	T1	N0M0	3
475MM	77	F	Cau	malignant melanoma, NOS	face	T3	N0M0	4
476MM	87	F	Cau	spindle cell melanoma	upper limb & shoulder	T3	N0M0	4
477MM	82	M	Cau	malignant melanoma, NOS	scalp & neck	T2	N0M0	3
478MM	78	F	Cau	epithelioid melanoma	face	T3	N0M0	4
480MM	59	M	Cau	malignant melanoma, NOS	upper limb & shoulder	T2	N0M0	3
481MM	85	M	Cau	malignant melanoma, NOS	upper limb & shoulder	T3	N0M0	4
482MM	66	M	Cau	epithelioid melanoma	face	T3	N0M0	4
483MM	85	F	Cau	epithelioid melanoma	trunk	T4	N0M0	5
484MM	70	F	Cau	malignant melanoma, NOS	upper limb & shoulder	T1	N0M0	3
511MM	69	M	Cau	epithelioid melanoma	skin, NOS	T3	N1M0	4
512MM	45	M	Cau	epithelioid melanoma	trunk	T4	N0M0	3

Table 7

Characteristics	Melanoma (%)	Nevi (%)	Normal skin (%)
Mean Age	65.51 ± 14.55	33.17 ± 15.60	n/a
Gender			
Female	22 (48.9)	9 (50)	6
Male	23 (51.1)	0 (50)	1
Anatomical location			
Face	5 (11.1)	3 (17)	
Scalp and neck	4 (8.0)	1 (6)	
Trunk	9 (20)	10 (55)	6 (86)
Upper limb and shoulder	17 (37.8)		
Lower limb and hip	6 (13.3)	4 (22)	
Skin, NOS	4 (8.9)		1 (14)
Histological diagnosis			
Epitheloid cell	20 (44.4)		
Spindle cell	7 (15.6)		

Malignant melanoma NOS	18 (40)		
Compound nevus		8 (44)	
Intradermal nevus		5 (28)	
Atypical nevus		2 (11)	
Benign nevus, NOS		3 (17)	
Normal skin			7 (100)
T stage (thickness)			
T1	11 (24.4)		
T2	14 (31.1)		
T3	16 (35.6)		
T4	4 (8.9)		
N stage			
N0	42 (93.3)		
N1	3 (6.7)		
M stage			
M0	44 (97.8)		
M1	1 (2.2)		
Clark level			
2	9 (20)		
3	16 (35/6)		
4	12 (26.7)		
5	2 (4.4)		
n/a	6 (13.3)		

Example 2

RNA Isolation from malignant melanoma and benign skin nevi samples

Qiagen RNeasy™ Mini Kit (QIAGEN Inc., Valencia, CA) was used, with a modified protocol to minimize the residual melanin in the RNA sample. For melanocyte containing tissues, four replicate tissue samples derived from individual patient each weighed approximately 5 mg and were used and processed separately. Tissue samples were homogenized in 1.0 ml RLT buffer (QIAGEN) containing 10 µl β-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) by a mechanical homogenizer (UltraTurrex T8, IKA-Werke, Staufen, Germany). After homogenization, samples were loaded onto QIAGEN RNeasy™ columns and followed by centrifugation. After discarding the flow-through, 700 ml of RW1 buffer was added; the column was kept for 5 min at room temperature and then centrifuged. This step was repeated 3 times. Then the standard QIAGEN RNeasy™ Mini Kit protocol was followed. To remove RNA from the silica gel membrane, a two-step elution was performed. The total RNA derived from the same individual patient tissue was pooled and used for further analysis.

Standard Trizol protocol was used for RNA isolation from tissues that do not contain a significant proportion of melanocytes. Tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA). After centrifugation the top liquid phase was collected and total RNA was precipitated with isopropyl alcohol at -20^{0}C. RNA pellets were washed with 75% ethanol, resolved in water and stored at -8O^{0}C until use. RNA quality was examined with an Agilent 2100 Bioanalyzer RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA).

Labeled cRNA was prepared and hybridized with the high-density oligonucleotide array Hul 33A Gene Chip (Affymetrix, Santa Clara, CA) containing a total of 22,000 probe sets according to the standard manufacturer protocol. Arrays were scanned using Affymetrix protocols and scanners. For subsequent analysis, each probe set was considered as a separate gene. Expression values for each gene were calculated by using Affymetrix Gene Chip analysis software MAS 5.0. All chips met three quality control standards: "present" call was greater than 35%, scale factor was smaller than 12 when scaled to a target intensity of 600, and background level was less than 150. Lower than usual percent of "present" calls cut-off was chosen because it is difficult to isolate RNA from skin cells (Hipfel et al. (1998)) resulting in lower overall gene expression levels.

Example 3

Data Analysis

Gene expression data were filtered to include only genes called "present" in 2 or more samples. This filter was used to remove genes that did not change expression in the samples. Of the 22,000 genes presented on the array, 15,795 passed this filter and were used for hierarchical clustering. Prior to clustering, each gene expression signal was divided by the median expression in al samples in the data set. This standardization step minimized the effect of the magnitude of gene expression and group together genes with similar expression patterns in the clustering analysis. Average linkage hierarchical clustering using Pearson correlation was performed on both the genes and the samples using GeneSpring 6.1.

In order to identify differentially expressed genes, we compared the melanoma samples to the benign nevi and the normal skin samples separately. The first analysis

consisted of the 45 melanoma and 7 normal skin samples; the second analysis consisted of 45 melanoma and 18 nevi samples. These two datasets were analyzed separately in following procedures as shown in Fig. 1. Significance analysis of microarray (SAM; Tusher et al. (2001)) and Student T-test were used in gene selection. Parameters for SAM were set as Δ=2.5 and fold change = 2.0 with 1,000 permutations. FDR was 1%. There were no missing data and the default random number was used. Next percentile analysis was conducted. For up-regulated genes the 30%ile in melanoma samples was compared to the maximum of the normal samples, or that of nevi samples. Student T-test with Bonferroni correction was also performed with cut-off $p<0.05$ in order to ensure that the selected genes had significant differential expression between the two groups of the samples. As a final step, we identified common genes between the melanoma/benign and melanoma/normal gene lists resulting in the single list of genes upregulated in melanoma shown in Figure 1 where the 439 common genes correspond to SEQ ID NOs: 29-467 as described in Table 15 with the results shown in Table 8.

Table 8

PSID	Median Expression in Melanoma	Fold Change (Cancer vs Benign)	Fold Change (Cancer vs skin)
200078_s_at	3954	2	3
200601_at	9254	2	7
200612_s_at	2396	2	5
200644_at	7240	3	6
200660_at	14659	3	4
200707_at	3153	2	51
200736_s_at	7305	3	3
200737_at	2423	2	2
200783_s_at	1028	2	2
200825_s_at	3746	3	3
200827_at	1593	2	2
200837_at	5817	2	5
200838_at	19225	8	17
200839_s_at	28353	5	7
200859_x_at	9665	3	7
200910_at	7780	2	4
200950_at	8419	3	7
200954_at	3132	2	6
200966_x_at	27388	2	3
200967_at	6154	2	4
200968_s_at	5587	2	7

200972_at	8943	3	3
201038_s_at	1480	2	2
201051_at	5439	2	5
201105_at	33285	4	5
201106_at	7546	2	4
201188_s_at	1730	2	8
201189_s_at	3870	3	3
201195_s_at	6005	4	18
201202_at	1860	3	2
201251_at	23965	5	11
201252_at	901	2	3
201271_s_at	1648	2	5
201291_s_at	601	3	42
201313_at	2414	5	6
201346_at	3359	2	2
201393_s_at	2166	2	5
201416_at	4845	4	4
201417_at	2905	2	4
201470_at	18525	4	4
201474_s_at	2471	5	22
201485_s_at	1398	2	10
201486_at	1105	2	3
201536_at	2441	2	4
201614_s_at	651	2	4
201660_at	4713	7	3
201661_s_at	3336	6	5
201662_s_at	2693	5	3
201670_s_at	3047	2	12
201714_at	993	2	3
201765_s_at	5475	4	5
201792_at	3897	3	4
201804_x_at	5609	3	3
201819_at	2575	5	3
201850_at	11103	10	20
201880_at	1833	2	3
201910_at	3854	4	4
201911_s_at	2154	3	4
201931_at	2578	3	2
201954_at	25901	9	15
201976_s_at	7697	2	6
202069_s_at	855	2	3
202070_s_at	2490	4	5
202111_at	1380	3	9
202154_x_at	10260	3	4
202185_at	8493	4	6
202188_at	1269	3	9
202219_at	5630	3	7

202224_at	2650	3	4
202225_at	1534	2	2
202260_s_at	7877	6	6
202295_s_at	15129	6	4
202329_at	3438	4	4
202367_at	898	2	2
202370_s_at	4669	3	2
202478_at	7922	3	11
202503_s_at	2424	3	5
202589_at	3494	2	6
202603_at	3772	3	2
202705_at	1058	4	4
202737_s_at	2865	3	4
202779_s_at	3400	9	55
202785_at	1134	2	6
202862_at	2540	6	4
202898_at	4736	4	63
202954_at	1357	2	2
202958_at	2401	4	3
202961_s_at	19648	4	5
202986_at	2052	3	28
203011_at	1346	2	2
203022_at	1068	2	2
203069_at	560	15	13
203071_at	958	19	3
203094_at	968	2	2
203145_at	513	2	2
203167_at	2523	3	5
203217_s_at	6416	3	5
203234_at	725	3	3
203256_at	7799	5	31
203262_s_at	2482	2	2
203300_x_at	5278	3	16
203315_at	3273	2	2
203366_at	847	2	2
203396_at	3946	2	3
203452_at	579	4	12
203456_at	1132	2	3
203502_at	657	3	3
203518_at	2943	3	7
203554_x_at	5056	3	4
203557_s_at	839	2	2
203570_at	2553	4	15
203590_at	3093	3	7
203643_at	1380	3	8
203663_s_at	9720	5	3
203668_at	1963	2	3

203693_s_at	588	3	3
203695_s_at	1548	6	3
203723_at	4087	5	9
203729_at	9154	2	14
203730_s_at	517	3	3
203731_s_at	691	3	2
203775_at	1251	4	3
203827_at	3380	11	8
203878_s_at	2036	8	5
204014_at	7184	10	46
204015_s_at	2207	5	15
204033_at	2154	7	7
204092_s_at	664	3	3
204099_at	2496	6	6
204170_s_at	1362	3	4
204197_s_at	2889	3	4
204198_s_at	4024	3	3
204202_at	875	3	3
204228_at	1299	2	3
204244_s_at	538	4	4
204247_s_at	689	3	27
204252_at	5294	5	7
204262_s_at	1003	4	5
204423_at	689	4	3
204436_at	3113	3	3
204458_at	908	3	3
204467_s_at	1910	3	6
204584_at	9677	21	15
204585_s_at	805	13	17
204647_at	1693	3	3
204654_s_at	3894	4	25
204709_s_at	312	24	16
204778_x_at	537	2	4
204779_s_at	1641	4	4
204857_at	2736	3	297
204932_at	241	3	3
204973_at	1478	3	7
204995_at	496	4	6
205051_s_at	3875	4	3
205142_x_at	937	2	3
205169_at	267	2	7
205373_at	579	10	8
205376_at	815	2	3
205405_at	2302	4	11
205447_s_at	567	13	5
205458_at	3288	6	8
205566_at	2684	7	8

205591_at	1190	5	3
205681_at	1316	8	12
205690_s_at	9179	8	9
205691_at	226	7	5
205717_x_at	8127	3	3
205813_s_at	430	11	9
205937_at	301	7	5
205945_at	772	2	4
205996_s_at	909	2	3
206128_at	364	3	3
206307_s_at	534	8	4
206332_s_at	4671	2	2
206397_x_at	1436	6	43
206441_s_at	3681	6	31
206462_s_at	9953	53	24
206503_x_at	419	8	9
206617_s_at	898	4	19
206630_at	23194	3	46
206688_s_at	2989	3	2
206696_at	6446	7	191
206777_s_at	683	4	7
206864_s_at	421	5	5
206976_s_at	3375	4	3
207038_at	1986	9	47
207060_at	497	4	5
207144_s_at	593	17	24
207163_s_at	3217	3	10
207183_at	230	6	6
207592_s_at	350	5	14
207614_s_at	2139	2	6
207622_s_at	882	2	16
207828_s_at	997	3	3
208002_s_at	3142	3	7
208089_s_at	1374	3	6
208308_s_at	12282	4	9
208540_x_at	5257	2	2
208644_at	2242	2	3
208657_s_at	1547	2	4
208677_s_at	5414	3	14
208696_at	7351	4	3
208710_s_at	1112	3	44
208723_at	4402	3	6
208744_x_at	1673	4	49
208837_at	3997	2	3
208916_at	1630	3	5
208928_at	1439	4	7
208956_x_at	7772	3	2

208974_x_at	6025	2	6
208975_s_at	1085	2	3
209015_s_at	1739	4	4
209036_s_at	8944	3	3
209053_s_at	269	7	10
209072_at	6299	4	18
209079_x_at	12870	3	3
209081_s_at	3160	3	2
209123_at	4686	4	3
209132_s_at	4385	5	12
209172_s_at	268	3	3
209197_at	820	3	3
209198_s_at	491	3	3
209247_s_at	1486	2	2
209254_at	1384	4	7
209255_at	4283	6	8
209256_s_at	4949	8	6
209283_at	12529	5	3
209345_s_at	1678	2	2
209407_s_at	1461	2	6
209515_s_at	5827	5	15
209773_s_at	1243	3	5
209825_s_at	765	2	3
209827_s_at	4884	7	7
209828_s_at	1146	4	5
209848_s_at	32959	7	74
209875_s_at	3038	21	12
209932_s_at	7126	3	5
210052_s_at	1085	3	6
210073_at	337	4	6
210111_s_at	7841	5	3
210127_at	391	2	9
210854_x_at	2100	2	6
210926_at	574	2	3
210948_s_at	396	2	4
210951_x_at	2501	2	13
211013_x_at	498	8	13
211052_s_at	1399	3	2
211066_x_at	12431	2	2
211373_s_at	2063	5	6
211564_s_at	1992	3	2
211752_s_at	2183	2	2
211759_x_at	5674	2	3
211833_s_at	502	2	28
212000_at	339	3	14
212070_at	13437	2	4
212081_x_at	1457	2	4

212119_at	4415	2	5
212178_s_at	2976	3	14
212193_s_at	3646	3	10
212247_at	1716	3	3
212285_s_at	4252	2	4
212312_at	1234	4	2
212338_at	1598	3	4
212402_at	3019	3	4
212472_at	1987	5	5
212473_s_at	3747	5	4
212512_s_at	1441	3	2
212520_s_at	2188	2	4
212552_at	2611	2	2
212715_s_at	1085	3	4
212739_s_at	2736	2	3
212744_at	959	3	5
212745_s_at	376	2	15
212793_at	3123	4	5
212796_s_at	2511	2	2
213002_at	1439	2	3
213007_at	968	3	4
213008_at	1086	6	10
213096_at	924	3	3
213131_at	1392	3	3
213169_at	4028	5	4
213215_at	1926	4	3
213217_at	5848	10	6
213241_at	9479	4	23
213274_s_at	18263	12	26
213275_x_at	17604	7	3
213330_s_at	1233	2	7
213333_at	1845	2	3
213338_at	932	6	4
213392_at	1022	2	2
213474_at	723	2	3
213496_at	2322	3	7
213573_at	1643	2	3
213587_s_at	10416	18	9
213638_at	1827	26	102
213670_x_at	1959	2	4
213720_s_at	2248	2	3
213746_s_at	4187	3	14
213836_s_at	2605	8	5
213895_at	1279	3	4
213960_at	11768	80	29
214023_x_at	1602	7	9
214068_at	2148	9	10

214104_at	814	2	3
214201_x_at	746	2	3
214581_x_at	510	5	6
214614_at	542	10	9
214632_at	358	2	2
214656_x_at	2977	2	2
214687_x_at	26310	2	3
214708_at	249	2	3
214710_s_at	575	2	3
214714_at	2366	4	9
214717_at	471	4	4
214752_x_at	6462	3	4
214778_at	311	3	27
214841_at	913	9	11
214893_x_at	214	10	9
214896_at	3071	8	11
215025_at	2365	149	93
215115_x_at	12421	34	15
215126_at	4940	8	19
215155_at	505	6	4
215311_at	10093	86	30
215812_s_at	1176	3	13
215836_s_at	9406	3	3
216194_s_at	5011	3	3
216973_s_at	1732	6	4
217033_x_at	10961	21	19
217104_at	317	6	3
217226_s_at	2191	3	3
217297_s_at	838	3	21
217377_x_at	12402	27	18
217419_x_at	2742	3	5
217624_at	349	21	20
217799_x_at	1724	2	11
217827_s_at	4762	2	2
217867_x_at	9024	3	9
217871_s_at	19519	3	11
217891_at	1271	2	3
218009_s_at	1557	4	3
218030_at	1316	2	3
218074_at	3594	2	4
218143_s_at	4007	3	5
218151_x_at	1384	2	2
218152_at	1440	2	3
218161_s_at	941	5	5
218175_at	3563	3	2
218330_s_at	3853	7	4
218349_s_at	588	3	14

218359_at	796	3	5
218376_s_at	1931	4	4
218447_at	2209	2	3
218542_at	409	3	6
218564_at	433	2	4
218608_at	627	3	4
218678_at	11356	14	20
218774_at	1061	2	5
218786_at	732	3	2
218824_at	1351	3	5
218839_at	1996	38	7
218856_at	3199	5	4
218888_s_at	906	6	5
218931_at	911	3	2
218952_at	2661	7	7
218956_s_at	1840	4	3
218980_at	1627	3	8
218996_at	1859	4	6
219011_at	113	4	3
219039_at	1852	2	5
219040_at	480	3	10
219041_s_at	3435	4	2
219051_x_at	1127	3	8
219066_at	621	4	2
219143_s_at	3618	9	13
219148_at	426	2	3
219152_at	365	13	10
219219_at	859	2	3
219361_s_at	1033	3	7
219372_at	376	2	2
219408_at	421	3	36
219478_at	5485	80	18
219491_at	547	3	12
219522_at	822	4	3
219537_x_at	411	3	6
219555_s_at	402	12	21
219578_s_at	1419	17	26
219634_at	686	3	15
219637_at	355	3	3
219703_at	378	3	3
219742_at	409	3	9
219895_at	528	6	3
219933_at	1399	2	2
220116_at	748	5	8
220155_s_at	5010	5	6
220178_at	5915	9	15
220454_s_at	581	2	2

220864_s_at	8416	3	3
220948_s_at	11794	2	3
220973_s_at	497	4	3
220974_x_at	2540	2	2
220980_s_at	3598	3	2
221059_s_at	2438	5	3
221483_s_at	9194	3	3
221484_at	3834	3	3
221538_s_at	3971	3	3
221558_s_at	2356	3	5
221577_x_at	4897	28	38
221641_s_at	1199	2	4
221688_s_at	3740	2	4
221710_x_at	728	2	2
221732_at	931	3	2
221759_at	1261	4	21
221797_at	430	2	7
221799_at	1601	5	3
221815_at	3293	17	144
221882_s_at	1144	5	6
221902_at	2491	4	4
221909_at	243	22	17
221962_s_at	1132	2	6
222116_s_at	4208	2	4
222153_at	445	3	8
222155_s_at	1264	3	10
222175_s_at	2415	3	6
222196_at	224	3	6
222199_s_at	2152	2	3
222206_s_at	383	4	12
222212_s_at	3715	3	4
222231_s_at	2724	3	2
222234_s_at	754	4	12
222240_s_at	1331	3	3
222294_s_at	2193	3	5
32811_at	4194	2	3
40560_at	1629	3	5
44783_s_at	8503	14	6
46665_at	7835	4	3
55093_at	3032	4	4
63825_at	10096	14	74
87100_at	737	6	70

We selected a short list of genes with at least 10-fold over-expression in melanoma as compared to the benign specimens. The complete array dataset has been submitted to the NCBI/Genbank GEO database (series entry pending).

Hierarchical clustering revealed four distinct clusters (Fig. 2). Two clusters consisted of majority of the melanoma samples (43 out of 45); the third cluster included the majority of benign nevi samples (15 out of 18) and the fourth contained all 7 normal skin specimens. Melanoma samples themselves formed two clusters with 35 samples in one cluster and 10 samples in the other. Samples that formed the small cluster represented epithelioid melanoma only, visually contained less melanin and demonstrated higher expression of PRAME and MIA genes ($p<0.05$). The few stage III and IV tumors were all grouped in the small cluster. The large cluster showed higher expression of NTRK3 and nestin (NES) ($p<0.05$). All melanoma and benign nevi samples demonstrated equally high expression of known melanocyte markers such as tyrosinase and MART-I, confirming that there is comparable melanocyte content in these samples. Our data indicate that melanoma, benign nevi and normal skin samples have distinct gene expression profiles and can be separated on molecular basis. Selected genes that were highly expressed in melanoma and their associated functional categories are summarized in Table 9.

Table 9

psid	Name	Median expression in melanoma	Median expression in benign/normal	Fold change
Neural system development and function				
215025_at	NTRK3	2365.1	19.9	118.8
204709_s_at	KNSL5	311.8	13.5	23.1
204585_s_at	L1CAM var 1	805.2	49	16.4
218678_at	NES	11355.5	703.6	16.1
202260_s_at	STXBP1	7877.1	1312	6.0
204995_at	p35	496.3	89.2	5.6
208308_s_at	GP1	12281.7	2238.2	5.5
201340_s_at	ENC1	390.8	74.9	5.2
209072_at	MBP	6299.1	1215.9	5.2
Cell movements				
214614_at	HOXB9	541.6	54.4	10.0
205447_s_at	MAP3K12	566.5	76.5	7.4
Tissue morphology				
206397_x_at	GDF1	1436.1	130.1	11.0

205458_at	MC1R	3287.6	458.1	7.2
Cancer cell invasion				
213274_s_at	CSTTB	18262.9	1261.4	14.5
208677_s_at	BSG	5413.8	1088	5.0
Cell cycle control				
219578_s_at	CPEB1	1418.5	75.6	18.8
207144_s_at	CITED1	593.4	33.5	17.7
204252_at	CDK2	5293.7	869.3	6.1
211373_s_at	PSEN2	2063.3	403.2	5.1
Cell death				
221577_x_at	PLAB	4896.9	173.9	28.2
205681_at	BCL2A1	1316.4	135.1	9.7
Unknown				
204545_at	PEX6	379.1	23.9	15.9
201850_at	CAPG	11103.2	725.6	15.3
204014_at	DUSP4	7183.6	601.3	11.9
202779_s_at	E2-EPF	3400	323.3	10.5
201954_at	ARPC1B	25900.7	2470	10.5
209848_s_at	me20m	32958.9	3778.4	8.7
213112_s_at	SQSTM1	260.4	33.9	7.7
218952_at	SAAS	2660.7	368.7	7.2
204099_at	SMARCD3	2496.2	428.2	5.8
206999_at	IL12RB2	354	61.3	5.8
201251_at	PKM2	23964.7	4228.2	5.7
202185_at	PLOD3	8493.2	1541.7	5.5

Example 4

Identification of genes differentially expressed in melanoma

A total of 70 gene expression profiles were used for analysis. The he median percentages of "present calls" for melanoma, benign and normal sample groups were 43.8%, 46.9% and 41.7%. Sixty microarrays (86%) had scaling factors within 3-fold range of the minimum value. Ten chips with the scaling factors more than 3 were equally distributed between the sample categories, melanoma, benign and normal.

Unsupervised hierarchical clustering result revealed a distinct separation of the melanoma, benign nevi and normal skin samples (Fig. 2). We observed four clusters, including two clusters consisting of majority of the melanoma samples (43 out of 45), the third cluster contained all 7 normal skin, 3 benign nevi and 2 melanoma specimens and the fourth cluster, that included 14 of the 18 benign nevi samples. Source of the samples did not affect clustering. Specimens originated from different sources were clustered together according the sample type (melanoma, benign or

normal). To further test the stability of the clustering patterns, we used an alternative cut-off on gene filtering prior to the cluster analysis. Specifically, we retained genes that have at least 10% "present" calls in each of the melanoma, benign nevi and skin samples. With this cut-off, we obtained 15, 306 genes and repeated hierarchical clustering. The cluster pattern on the patient samples was the same as the one from the 15,795 from the 2 "present" calls, confirming clustering stability.

The single nevi sample that clustered with the melanoma samples is an atypical nevi (moderate degree) sample with no melanoma *in-situ* present. All three nevi samples that clustered with normal skin are compound nevi samples and one of them has melanocyte content lower than the other nevi specimens. The melanoma samples themselves formed two clusters with 34 samples in the large and 9 samples in the smaller cluster. Samples that formed the small cluster represented epithelioid melanoma only and visually contained less melanin. The few stage III and IV tumors, used in our study, were all grouped in the small cluster. The large cluster was composed from epithelioid, spindle cell and melanoma of mixed histology specimens with more significant presence of melanin. The large cluster included Stage I and Stage II specimens only.

Distinct gene clusters were found in association to melanoma. This can be characterized by up-regulated (Fig. 2, A, B, C) and down-regulated (Fig. 2, E) genes in the melanoma samples. At the same time, melanoma and benign nevi samples demonstrated high expression of known melanocyte markers, such as MART-I (Fig. 3, D) confirming a comparable content of melanocyte in these samples and inability of melanocyte specific markers to differentiate them. Our data indicate that melanoma, benign nevi and normal skin samples have distinct gene expression profiles and can be separated on their molecular basis.

In order to identify genes upregulated in malignant melanoma, we applied SAM in combination with t-test with Bonferroni correction and percentile analysis (Fig. 1). Bonferroni-adjusted t-test and percentile analyses were used to address the multiple testing issue and the heterogeneity of the tumor samples, respectively. As the result of these analyses, 439 genes were selected and are summarized in Table 15 as SEQ ID NOs: 29-467. Out of 439 genes up-regulated in melanoma, we selected a short list of

33 genes that had more than 10-fold over-expression in the melanoma samples than that of the benign specimens. These include many genes with known association with malignant melanoma such as NTRK3 (Xu et al. (2003)), LICAM (Fogel et al. (2003); and Thies et al. (2002)), me20m (Adema et al. (1994)), as well as novel genes. Genes with more than 10-fold overexpression in melanoma are presented in Table 10.

Table 10

Psid	Description	Median Exp Melanoma	Fold change (Can v Benign)	Fold change (Can v skin)
215025_at	NTRK3	2365	149	93
215311_at	EUROIMAGE 21920	10093	86	30
213960_at	EUROIMAGE 51358	11768	80	29
219478_at	WFDC1	5485	80	18
218839_at	HEY1	1996	38	7
215115_x_at	TEL oncogene	12421	34	15
221577_x_at	PLAB	4897	28	38
217377_x_at	ETV6-NTRK3 fusion	12402	27	18
213638_at	PHACTR1	1827	26	102
204709_s_at	KNSL5	312	24	16
221909_at	Hyp protein FLJ14627	243	22	17
204584_at	L1CAM	9677	21	15
209875_s_at	SPP1	3038	21	12
217624_at	PDAP1	349	21	20
203071_at	SEMA3B	958	19	3
213587_s_at	C7ORF32	10416	18	9
221815_at	ABHD2	3293	17	144
219578_s_at	CPEB1	1419	17	26
207144_s_at	CITED1	593	17	24
203069_at	SV2A	560	15	13
218678_at	NES	11356	14	20
219152_at	PODXL2	365	13	10
205447_s_at	MAP3L12	567	13	5
213274_s_at	CTSB	18263	12	26
219555_s-at	BMO39	402	12	21
203827_at	WIPI49	3380	11	8
205813_s_at	MAT1A	430	11	9
201850_at	CAPG	11103	10	20
205373_at	CTNNA2	579	10	8
214614_at	HLXB9	542	10	9
213217	ADCY2	5848	10	6
204014_at	DUSP4	7184	10	46
214893	HCN2	214	10	9

We further selected three genes over-expressed in melanoma, including NTRK3, PLAB, LICAM, for quantitative real-time RT-PCR validation of the microarray results (Fig. 3). PLAB is a novel gene, whose differential expression in melanoma was not reported before at our best knowledge. For LICAM and NTRK3, differential

expression in melanoma was demonstrated at protein level only. Xu et al. (2003); Fogel et al. (2003); and Thies et al. (2002). Moreover, we identified PLAB and LlCAM as the best combination, on complementary basis, to separate melanoma from benign/normal tissues in our study. GPlOO is known as a melanoma specific marker and was selected as positive control. For the RT-PCR assay we used a panel of 14 primary melanoma, 7 benign nevi and 5 normal skin samples, isolated from the same tissues as used for the microarray study. The expression value of each gene was normalized to the housekeeping control gene PBGD. The correlation coefficients between the RT-PCR and the microarray results for LlCAM, NTRK3, PLAB and gplOO are 0.79, 0.86, 0.87 and 0.88, respectively. This result indicates that the RT-PCR results are highly consistent with the microarray data.

Example 5

Pathway Analysis **of** Differentially **Expressed Genes**

Functional analysis of genes differentially expressed in melanoma was performed using Ingenuity™ Pathway Analysis Software Application (Ingenuity, Mountain View, CA). Functional categories or canonical pathways that have p-value of less than 0.05 were selected. Specificity of canonical pathways identification was tested using randomly selected genes.

In order to gain further insight into a potential mechanism that differentiates melanoma from benign and normal tissue, we used Ingenuity pathway analysis software to identify canonical pathways associated with melanoma. The results analysis revealed that many of the genes in amyloid processing were up-regulated in the melanoma samples. To verify specificity of our observation, we selected three random lists of genes from Affymetrix HuI 33A microarray and subjected them to Ingenuity pathway analysis. None of these lists produced a significant association to amyloid processing or any other canonical pathways. To confirm the activation of this canonical pathway in melanoma, gene expression data for all the genes in the pathway were retrieved. Fold-change and p-value of differential expression between melanoma and benign/normal tissues were calculated. Out of the 34 genes included in the amyloid processing pathway (Esler et al. (2001); and Giancotti et al. (1999)), 25 demonstrated up-regulation trend and for 19 of them (56%), differential expression was statistically significant (p-value< 0.05; Fig 4). As the additional control, we

randomly selected two metabolic pathways with a similar number of genes. Out of the 63 genes in alanine synthesis pathway, 8 of them (13%) showed significant up-regulation with p-value less than 0.05. Out of the 47 genes in histidine synthesis pathway, only 2 genes (4%) were found using the same criteria. For the first time, our data strongly indicated that activation of the amyloid processing pathway is involved in malignant melanoma.

Example 6
RT-PCR Validation of Microarray **Results**

Ten microgram total RNA from each sample was treated with DNase I and reverse-transcribed with oligo (dT) primer using Superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). A control gene PBGD was previously tested and reported as a housekeeping gene. Vandesompele et al. (2003). Primers and MGB-probes for me20m (gplOO), LlCAM, NTRK3, and the control gene PBGD were designed using Primer Express software (Applied Biosystems, Foster City, CA). The PLAB (MICl) gene probe was FAM-TAMRA based since sequences were inadequate to design MGB based probes. Primer/probe sequences were as follows:

Table 11

Description	Sequence	SEQ ID NO:
me20m forward	TGTGTCTCTGGCTGATACCAACA	983
me20m reverse	TTCTTGACCAGGCATGATAAGCT	984
me20m probe	(6-FAM) CTGGCAGTGGTCAGC	985
L1CAM forward	GCTGGGACTGGGAACAGAACT	10
L1CAM reverse	GGAGCAGAGATGGCAAAGAAA	11
L1CAM probe	(6-FAM) TCCCCACCATCTGCTGT	12
NTRK3 forward	GCCCCGGCACCCTTTA	16
NTRK3 reverse	AACCCTGCCAGTGGTGGAT	17
NTRK3 probe	(6-FAM) CAGATGGGTGTTTTC	18
PLAB forward	GGCAGAATCTTCGTCCGCA	4
PLAB reverse	GGACAGTGGTCCCCGTTG	5
PLAB probe	(6-FAM) CCCAGCTGGAGTTGCACTTGCGGCC(TAMRA)	6

PBGD forward	CTGCTTCGCTGCATCGCTGAAA	986
PBGD reverse	CAGACTCCTCCAGTCAGGTACA	987
PBGD probe	(6-FAM) CCTGAGGCACCTGGAAGGAGGCTGCAGTGT(TAMRA)	988

All primers and probes were tested for optimal amplification efficiency above 90%. The standard curve was composed of six 10-fold dilutions of target gene PCR product with copy numbers ranging from 10 to 10^6. RT-PCR amplification was carried out in a 20μl reaction mix containing 50ng template cDNA, 2 x TaqMan® universal PCR master mix (12.5μl) (Applied Biosystems, Foster City, CA), 50OnM forward and reverse primers, and 25OnM probe. Reactions were run on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The cycling conditions were: 2 min of AmpErase UNG activation at $5O^0C$, 10 min of polymerase activation at 95^0C and 50 cycles at 95^0C for 15 sec and annealing temperature ($6O^0C$) for 60 sec. In each assay, a standard curve and a no-template control along with template cDNA were included in duplicate for both the gene of interest and the control gene. The relative quantity of each target gene was represented as ΔCt, which is equal to Ct of the target gene subtracted by Ct of the control gene.

To confirm the melanoma specific genes identified by the microarray analysis, four genes (LlCAM, NTRK3, PLAB and gplOO) were selected for quantitative real-time RT-PCR validation (Fig. 4). The expression value of each gene was normalized to housekeeping control PBGD. The correlation coefficient between the RT-PCR and the microarray results for LlCAM, NTRK3, PLAB and gplOO are 0.79, 0.86, 0.87 and 0.88, respectively, indicating that the RT-PCR results are highly consistent with the microarray data.

Example 7
One-step qRTPCR Assays Using RNA-specific Primers and Cutoff Establishment

Evaluation of expression of selected genes was carried out with one-step RT-PCR with RNA from primary melanoma, benign nevi, normal skin, melanoma LN metastasis and melanoma-free lymph nodes. Beta-actin was used as a housekeeping gene to control for the input quantity and quality of RNA in the reactions. DNase

treatment was not used. Instead, primers or probes were designed to span an intron so they would not report on genomic DNA. Eight ng of total RNA was used for the RT-PCR. The Total RNA was reverse transcribed using 4OX Multiscribe and RNase inhibitor mix contained in the TaqMan® One Step PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, CA). The cDNA was then subjected to the 2x Master Mix without UNG and PCR amplification was carried out on the ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in the 384-well block format using a 10 µl reaction size. The primer and probe concentrations were 4 µM and 2.5 µM, respectively. The reaction mixture was incubated at 48°C for 30 min for the reverse transcription, followed by a Amplitaq activation step of 95^{0}C for 10 min and finally 40 cycles of 95°C for 15 sec denaturing and 60°C for 1 min anneal and extension. On each plate a standard curve is generated from 8 pg to 80 ng and when the R2 value was greater than 0.99 the Cycle Threshold (Ct) values were accepted.

Sequences used in the reactions were as follows, each written in the 5' to 3' direction.

Table 12

Description	Sequence	SEQ ID NO:
L1CAM Forward	CCACAGATGACATCAGCCTCAA	13
L1CAM Reverse	GGTCACACCCAGCTCTTCCTT	14
L1CAM probe	TGGCAAGCCCGAAGTGCAGTTCC	15
Tyrosinase Forward	CTTTAGAAATACACTGGAAGGATTTGCTA	1000
Tyrosinase Reverse	CATTGTGCATGCTGCTTTGA	1001
Tyrosinase probe	TCCACTTACTGGGATAGCGGATGCCTC	1002
MART1 Forward	ACTTCATCTATGGTTACCCCAAGAA	1003
MART1 Reverse	TCCCAGCGGCCTCTTCA	1004
MART1 Probe	CACGGCCACTCTTACACCACGGC	1005
HMB45 Forward	CTTAAGGCTGGTGAAGAGACAAGTC	1006
gp100 Reverse	CAGGATCTCGGCACTTTCAATAC	1007
gp100 Probe	TCGATATGGTTCCTTTTCCGTCACCCTG	1008
PLAB Forward	ATTCGAACACCGACCTCGTC	1009

PLAB Reverse	CGCAGGTGCAGGTGGC	1010
PLAB Probe	GATACTCACGCCAGAAGTGCGGCT	1011

For each sample ΔCt=Ct (Target Gene) - Ct β-actin was calculated. ΔCt has been widely used in clinical RT-PCR assays and was chosen as a straightforward method. Cronin et al. (2004). T-test was performed on ΔCt between the melanoma and non-melanoma samples including both primary and LN samples. We then used ΔCt to construct two scores for each patient. One score was derived from a combination of 2 melanoma specific genes, PLAB and LlCAM; and the other score was derived from a combination of 3 conventional melanoma markers, tyrosinase, gplOO and MARTl . The score was defined as the weighted sum of ΔCt values of the tested genes with the corresponding *t* statistics as the weight. The two scores were normalized to have the same mean in order to compare them on the same scale.

We examined a combination of two highly overexpressed in melanoma genes, PLAB and LlCAM, in a variety of clinical tissue samples containing malignant melanocytes (primary melanoma and melanoma LN metastasis), benign melanocytes (benign skin nevi) and normal samples (normal skin and melanoma- free LN) by RT-PCR. The primary tissues were the same as those used for the microarray study while all the LN specimens were derived from independent patients. Conventional melanoma markers, such as tyrosinase, gplOO and MARTl, were also tested on the same samples as the controls because they are the most commonly used markers for the melanoma molecular assays in current clinical studies. Rimboldi et al. (2003); Abrahamsen et al. (2005); and Kammula et al. (2004). Calculated scores were presented on Fig. 4A for PLAB and LlCAM and on Fig. 4B for tyrosinase, gplOO and MARTl. The results demonstrated significant difference in expression of PLAB and LlCAM between malignant melanoma samples (primary and LN metastasis) and benign nevi and normal LN. In contrast, three conventional markers showed similar expression levels in benign and melanoma samples. To further demonstrate the ability of gene markers to separate benign and malignant tissues, we tested two cut¬ offs; first was set up as the highest score in primary normal samples and the second as the highest score in benign nevi samples. For each cut-off we estimated sensitivity and of the assay in the LN samples. With the cut-off determined on the normal

samples, the new markers and the conventional markers gave sensitivity of 90% and 83%, respectively. Using the cut-off determined on the benign samples, the sensitivity for the new and conventional markers were 88% and 42%. The results indicated that the new markers potentially have better abilities to differentiate tissues containing benign and malignant melanocytes.

Example 8
Multiplex Assay

Materials and methods

Each reaction was set up in a final volume of 25 µl containing the following:

forward primer	400 nM
reverse primer	500 nM
PLAB probe	150 nM
Tyrosinase probe	300 nM
LICAM probe	200 nM
PBGD probe	200 nM
Tth	5 U
Ab TP 6-25	1 µg
Glycerol	10%
Tris-HCl	31 mM
NaCl	4 mM
EDTA	0.004 mM
Tween-20	0.22%
NP-40	0.02%
DTT	0.04 mM
Potassium Hydroxide	20.5 mM
Bicine	50 mM
Potassium Acetate	115 mM
Albumin, bovine	5 µg
Trehalose	0.15 M
dNTP	0.2 mM ea
$MgCl_2$	0.5 mM
$MnSO_4$	3.5 mM
Primers	300 nM ea
Probes	200 nM ea

The primer and probe sequences are provided in Table 13.

Table 13

SEQ ID NO	Sequence 5'-3'	Function
43	gaacaccgacctcgtccc	PLAB Upper Primer
44	ggcggcccgagagata	PLAB Lower Primer
45	Fam-cgccagaagtgcggctgggat-BHQ1-tt	PLAB Probe
55	actcagcccagcatcattcttc	Tyr Upper Primer
56	atggctgttgtactcctccaatc	Tyr Lower Primer
57	Q570-cttctcctcttggcagattgtctgtagc BHQ2-tt	Tyr Probe
49	ccacagatgacatcagcctcaa	L1CAM Upper Primer
50	ggtcacacccagctcttcctt	L1CAM Lower Primer
51	CalRed-tggcaagcccgaagtgcagttcc-BHQ2-tt	L1CAM Probe
58	ccacacacagcctactttccaa	PBGD Upper Primer
59	tacccacgcgaatcactctca	PBGD Lower Primer
60	Q670-aacggcaatgcggctgcaacggcggaa-BHQ2-tt	PBGD Probe

The reactions are run with PLAB in Fam, Tyrosinase in Cy3, L1CAM in Texas Red, and PBGD in Cy5 channels. The cycling protocol used is described below and takes 30 min to complete.

95°C x 15 sec
65°C x 420 sec
40°cycles of:
95°C for 5 sec
62°C for 15 sec - fiuor read

The thresholds used are 30 in Fam, 20 in Cy3, 20 in Texas Red, and 20 in Cy5 channels. The thresholds employed in the Cy3 and Texas red channels can be lowered. The results obtained are summarized in Table 14.

Table 14
Best Marker Combinations

Markers	% Sensitivity (95% CI)	% Specificity (95% CI)
L1CAM + PLAB	82 (73-89)	96 (87-100)
Tyrosinase + ME20M (GP100)	63 (52-72)	100 (94-100)
L1CAM + PLAB + Tyrosinase	87 (79-93)	96 (87-100)

Ct cutoffs:

L1CAM	27
PLAB	29
Tyrosinase	23
ME20M (GP1OO)	23.5

Note: these data are benchmarked against H&E pathology only. The amplification efficiency in each of the 4 reactions is high and the reaction is also linear over 5 logs (as judged by the R2 value which is >0.99 in all cases). Therefore,

these data demonstrate a working 4 plex, rapid assay. These data suggest that PLAB is the primary marker and complementation, achieved with LlCAM, further increases sensitivity. If required, addition of tyrosinase as a third marker further complements LlCAM and PLAB and increases sensitivity. Tyrosinase can be dropped from the assay, if needed, without affecting the performance of the remaining markers.

Discussion

We performed gene expression profiling analysis of primary melanoma, benign nevi and normal skin tissue specimens in order to find melanoma specific gene markers for potential use in the LN molecular staging assay. Novel genes that are highly and differentially expressed in malignant melanoma samples were identified. Inclusion of benign nevi in the experimental design was key to our study. In contrast to normal skin, melanocyte content in benign nevi is close to that in melanoma. This was confirmed, in addition to histological assessment, by equally high expression level of conventional melanoma markers such as tyrosinase and MARTl in both melanoma and nevi tissue specimens. Similar cellular composition allowed us to monitor gene expression changes specifically associated with melanocyte malignant transformation, not just with melanocyte lineage differentiation. As the result, we identified novel genes specifically overexpressed in melanoma. One of the novel highly overexpressed in melanoma genes, prostate differentiation factor (PLAB, MICl), is a member of transforming growth factor-beta superfamily and also known to be associated with other malignancies. Bae et al. (2003); and Welsh et al. (2003). PLAB reduces cell adhesion (Yamauchi et al. (2003)), implicating its potential role in melanoma progression. Pathway analysis of the overexpressed genes in melanoma indicated that many of these genes belong to neural tissue functioning and development, suggesting that dedifferentiation of melanocytes and activation of the processes related to a pluripotent progenitor cell might be important for melanoma development and progression. Moreover, the analysis of canonical pathways showed that neural tissue associated amyloid processing is significantly modulated in melanoma. Amyloid processing (APP) pathway itself has not been associated with melanoma development and progression before. Many genes in the APP pathway, such as members of the β- and Y-secretase family (BACE2, PSEN2) also participate

in the Notch pathway and play a role of cleavage of integral membrane proteins in both Notch and APP. Esler et al. (2001). Notch suppresses differentiation and helps maintain neural crest stem cells in undifferentiated state (Gangemi et al. (2004)) and Notch's involvement in melanoma and, particularly, the role of Y-secretases is the focus of many studies. Hoek et al. (2004); Baldi et al. (2003); and Wilson et al. (2000).

We have compared our results to the recent study of Haqq et al (2005). In their work, cDNA microarray containing 20,862 probes was used to profile benign nevi, primary melanoma and metastatic melanoma specimens. The sample set included metastatic and primary melanoma and benign nevi. Similar clustering results that separated the benign nevi and the primary malignant melanoma tissues were found in their study. Common genes were reported in both studies that can discriminate melanoma from benign nevi including kinesin-like 5 (KNSL5), prostate differentiation factor (PLAB), CITED1, osteopontin (SPP1), cathepsin B (CSTB), cadherin 3 (CDH3), presenilin 2 (PSEN2).

Our results of the one-step RT-PCR assay demonstrated that novel melanoma specific gene PLAB and LICAM expressed not only in primary melanoma tissues but also in melanoma LN metastasis. Moreover, the ability to differentiate malignant melanoma from benign nevi made them better candidates than the conventional markers for the molecular test of melanoma diagnostics. With further validation in clinical studies, these genes could be developed as specific markers for a molecular staging assay to detect melanoma micrometastasis during sentinel lymph node (SLN) biopsy procedure. Another potential application of the genes is for diagnosis of melanocyte lesions with uncertain pathological features.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

Table 15 Sequence Descriptions, names and SEQ ID NOs:

1			PLAB	
2			L1CAM	
3			NTRK	
4				PLAB forward primer
5				PLAB reverse primer
6				PLAB probe
7				PLAB upper primer
8				PLAB lower primer
9				PLAB probe
10				L1CAM forward primer
11				L1CAM reverse primer
12				L1CAM probe
13				L1CAM upper primer
14				L1CAM lower primer
15				L1CAM probe
16				NTRK primer
17				NTRK primer
18				NTRK probe
19				Tyrosinase upper primer
20				Tyrosinase lower primer
21				Tyrosinase probe
22				PBGD upper primer
23				PBGD lower primer
24				PBGD probe
25				PLAB amplicon
26				L1CAM amplicon
27				Tyrosinase amplicon
28				PBGD amplicon
29	200078_s_at	BC005876		ATPase, H+ transporting, lysosomal
30	200601_at	U48734		non-muscle alpha-actinin
31	200612_s_at	NM_001282	AP2B1	adaptor-related protein complex 2, β 1
32	200644_at	NM_023009	MACMARCKS	macrophage myristoylated alanine-rich C kinase substrate
33	200660_at	NM_005620	S100A11	S100 calcium-binding protein A11
34	200707_at	NM_002743	PRKCSH	protein kinase C substrate 80K-H
35	200736_s_at	NM_000581	GPX1	glutathione peroxidase 1
36	200737_at	NM_000291	PGK1	phosphoglycerate kinase 1
37	200783_s_at	NM_005563	LAP 18	leukemia-assoc phosphoprotein p18
38	200825_s_at	NM_006389	ORP150	oxygen regulated protein (150kD)
39	200827._at	NM_000302	PLOD	procollagen-lysine, 2-oxoglutarate 5-dioxygenase
40	200837_at	NM_005745	DXS1357E	accessory proteins BAP31BAP29
41	200838_at	NM_001908	CTSB	cathepsin B
42	200839_s_at	NM_001908	CTSB	
43	200859_x_at	NM_001456	FLNA	filamin A, alpha
44	200910_at	NM_005998	CCT3	chaperonin containing TCP1, sub 3(γ)
45	200950_.at	NM_006409	ARPC1A	actin related protein 23 complex, sub 1A
46	200950_at	NM_006409	ARPC1A	
47	200966_x_at	NM_000034	ALDOA	aldolase A, fructose-bisphosphate
48	200967_at	NM_000942	PPiB	peptidylprolyl isomerase B
49	200968_s_at	NM_000942	PPIB	
50	200972_at	BC000704		tetraspan 3

51	201038_s_at	BE560202		putative HLA class II assoc protein I
52	201051_at	BE560202		putative HLA class II assoc protein I
53	201105_at	NM_002305	LGALS1	lectin, galactoside-binding, soluble, 1
54	201106_at	NM_002085	GPX4	glutathione peroxidase 4
55	201188_s_at	D26351	ITPR3	type 3 inositol 1,4,5-trisphosphate receptor
56	201189_s_at	NM_002224	ITPR3	
57	201195_s_at	AB018009		L-type amino acid transporter 1
58	201202_at	NM_002592	PCNA	proliferating cell nuclear antigen
59	201251_at	NM_002654	PKM2	pyruvate kinase, muscle
60	201252_at	NM_006503	PSMC4	proteasome 26S subunit, ATPase, 4
61	201271_s_at	NM_016732	RALY	RNA-binding protein transcript var 1
62	201291_s_at	NM_001067		topoisomerase (DNA) II alpha
63	201313_at	NM_001975	ENO2	enolase 2
64	201346_at	NM_024551	FLJ21432	hypothetical protein FLJ21432
65	201393_s_at	NM_000876	IGF2R	insulin-like growth factor 2 receptor
66	201416_at	NM_003107		SRY-box 4
67	201417_at	NM_003107		SRY-box 4
68	201470_at	NM_004832	GSTTLp28	glutathione-S-transferase like; glutathione transferase omega
69	201474_s_at	NM_002204	ITGA3	integrin, alpha 3 transcript variant a
70	201485_s_at	BC004892	RCN2	reticulocalbin 2, EF-hand calcium binding domain
71	201486_at	NM_002902	RCN2	
72	201536_at	AL048503		DKFZp586M1524
73	201614_s_at	NM_003707	RUVBL1	RuvB (E coli homolog)-like 1
74	201660_at		FACL3	fatty-acid-Coenzyme A ligase, long-chain 3
75	201661_s_at	NM_004457	FACL3	
76	201662_s_at	D89053		Acyl-CoA synthetase 3
77	201670_s_at	M68956	MARCKS 80K-L	myristoylated alanine-rich C-kinase substrate
78	201714_at	NM_001070	TUBG1	tubulin, gamma 1
79	201765_s_at	AL523158		hexosaminidase A
80	201792_at	NM_001129	AEBP1	AE-binding protein 1
81	201804_x_at	NM_001281	CKAP1	cytoskeleton-associated protein 1
82	201819_at	NM_005505	CD36L1	CD36 antigen-like 1
83	201850_at	NM_001747	CAPG	capping protein gelsolin-like
84	201880_at	NM_005744		ariadne (Drosophila) homolog, ubiquitin-conjugating enzyme E2-binding protein, 1
85	201910_at	BF213279	FARP1	RhoGEF & pleckstrin domain 1
86	201911_s_at	NM_005766	FARP1	
87	201931_at	NM_000126	ETFA	electron-transfer-flavoprotein, α polypeptide
88	201954_at	NM_005720	ARPC1B	actin related protein 23 com, sub 1A
89	201976_s_at	NM_012334	MYO10	myosin X
90	202069_s_at	AI826060	IDH3A	isocitrate dehydrogenase 3 alpha
91	202070_s_at	NM_005530	IDH3A	
92	202111_at	NM_003040	SLC4A2	solute carrier fam 4 anion exchanger mem 2
93	202154_x_at	NM_006086	TUBB4	tubulin, beta, 4
94	202185_at	NM_001084	PLOD3	procollagen-lysine, 2-oxoglutarate 5-dioxygenase

95	202188_at	NM_014669	KIAA0095	KIAA0095 gene product
96	202219_at	NM_005629	SLC6A8	solute carrier family 6, member 8
97	202224_at	NM_016823		v-crk avian sarcoma virus CT10 oncogene homolog
98	202225_at	NM_016823		v-crk avian sarcoma virus CT10 oncogene homolog
99	202260_s_at	NM_003165	STXBP1	syntaxin binding protein 1
100	202295_s_at	NM_004390	CTSH	cathepsin H
101	202329_at	NM_004383	CSK	c-src tyrosine kinase
102	202367_at	NM_001913	CUTL1	cut (Drosophila)-like 1
103	202370_s_at	NM_001755	CBFB	core-binding factor, β sub trans var 2
104	202478_at	NM_021643	GS3955	GS3955 protein
105	202503_s_at	NM_014736	KIAA0101	KIAA0101 gene product
106	202589_at	NM_001071	TYMS	thymidylate synthetase
107	202603_at	N51370		disintegrin and metalloproteinase domain 10
108	202705_at	NM_004701	CCNB2	cyclin B2
109	202737_s_at	NM_012321	LSM4	U6 snRNA-associated Sm-like protein
110	202779_s_at	NM_014501	E2-EPF	ubiquitin carrier protein
111	202785_at	NM_005001	NDUFA7	NADH dehydrogenase 1 α subcomplex, 7
112	202862_at	NM_000137	FAH	fumarylacetoacetate
113	202898_at	NM_014654	KIAA0468	KIAA0468 gene product
114	202954_at	NM_007019	UBCH10	ubiquitin carrier protein E2-C
115	202958_at	NM_002833	PTPN9	protein tyrosine phosphatase, non-receptor type 9
116	202961_s_at	NM_004889	ATP5J2	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2
117	202986_at	NM_014862	KIAA0307	KIAA0307 gene product
118	203011_at	NM_005536	IMPA1	inositol(myo)-1(or 4)-monophosphatase 1
119	203022_at	NM_006397	RNASEHI	ribonuclease HI, large subunit
120	203069_at	NM_014849	KIAA0736	KIAA0736 gene product
121	203071_at	NM_004636	SEMA3B	sema domain, Ig domain, short basic domain, secreted, 3B
122	203094_at	NM_014628	KIAA0110	gene predicted from cDNA
123	203145_at	NM_006461	DEEPEST	mitotic spindle coiled-coil related
124	203167_at	NM_003255	TIMP2	tissue inhibitor of metalloproteinase 2
125	203217_s_at	NM_003896	SIAT9	sialyltransferase 9
126	203234_at	NM_003364	UP	uridine phosphorylase
127	203256_at	NM_001793	CDH3	cadherin 3, type 1, P-cadherin (placental)
128	203262_s_at	NM_004699	DXS9928E	chromosome X 9928 expressed seq
129	203300_x_at	NM_003916	AP1S2	adaptor-related protein complex 1, sigma 2 subunit
130	203315_at	BC000103		NCK adaptor protein 2,
131	203366_at	NM_002693	POLG	polymerase (DNA directed), gamma
132	203396_at	NM_002789	PSMA4	proteasome subunit, α type, 4
133	203452_at	NM_012200	B3GAT3	beta-1,3-glucuronyltransferase 3
134	203456_at	NM_007213	JM4	JM4 protein
135	203502_at	NM_001724	BPGM	2,3-bisphosphoglycerate mutase
136	203518_at	NM_000081	CHS1	Chediak-Higashi syndrome 1
137	203554_x_at	NM_004219	PTTG1	pituitary tumor-transforming 1

138	203557_s_at	NM_000281	PCBD	6-pyruvoyl-tetrahydropterin synthasedimerization cofactor of hepatocyte nuclear factor 1 alpha
139	203570_at	NM_005576	LOXL1	lysyl oxidase-like 1
140	203590_a t	NM_006141	DNCLI2	dynein, cytoplasmic, light intermediate polypeptide 2
141	203643_at	NM_006494	ERF	Ets2 repressor factor
142	203663_s_at	NM_004255	COX5A	cytochrome c oxidase subunit Va
143	203668_at	NM_006715	MAN2C1	mannosidase, α, class 2C, mem 1
144	203693_s_at	NM_001949	E2F3	E2F transcription factor 3
145	203695_s_at	NM_004403	DFNA5	deafness, autosomal dominant 5
146	203723_at	NM_002221	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B
147	203729_at	NM_001425	EMP3	epithelial membrane protein 3
148	203730_s_at	BF196931	ZFP95	zinc finger protein homologous to Zfp95 in mouse
149	203731_s_at	NM_014569	ZFP95	
150	203775_at	NM_014251	SLC25A13	solute carrier family 25, member 13
151	203827_at	NM_017983	FLJ10055	hypothetical protein FLJ10055
152	203878_s_at	NM_005940	MMP11	matrix metalloproteinase 11
153	204014_at	NM_001394	DUSP4	dual specificity phosphatase 4
154	204015_s_at	BC002671	DUSP4	
155	204033_at	NM_004237	TRIP13	thyroid hormone receptor interactor 13
156	204092_s_at	NM_003600	STK15	serinethreonine kinase 15
157	204099_at	NM_003078	SMARCD3	SWISNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
158	204170_s_at	NM_001827	CKS2	CDC28 protein kinase 2
159	204197_s_at	NM_004350	RUNX3	runt-related transcription factor 3
160	204198_s_at	AA541630	RUNX3	
161	204202_at	NM_017604	KIAA1023	KIAA1023 protein
162	204228_at	NM_006347	USA-CYP	cyclophilin
163	204244_s_at	NM_006716	ASK	activator of S phase kinase
164	204247_s_at	NM_004935	CDK5	cyclin-dependent kinase 5
165	204252_at	M68520		cdc2-related protein kinase
166	204262_s_at	NM_000447	PSEN2	presenilin 2 transcript variant 1
167	204423_at	NM_013255	MKLN1	muskelin 1, intracellular mediator containing kelch motifs
168	204436_at	NM_025201	PP1628	hypothetical protein PP1628
169	204458_at	AL110209		DKFZp564A0122
170	204467_s_at	NM_000345	SNCA	synuclein, α transcript var NACP140
171	204584_at	AI653981	L1CAM	L1 cell adhesion molecule, MASA transcript var 1
172	204585_s_at	NM_000425	L1CAM	
173	204647_at	NM_004838	HOMER-3	Homer, neuronal imm early gene, 3
174	204654_s_at	NM_003220	TFAP2A	transcription factor AP-2 alpha
175	204709_s_at	NM_004856	KNSL5	kinesin-like 5
176	204778_x_at	AW102783	HOXB7	homeo box B7
177	204779_s_at	NM_004502	HOXB7	
178	204857_at	NM_003550	MAD1L1	MAD1-like 1
179	204932_at	BF433902		TNF receptor superfam, mem 11b
180	204973_at	NM_000166	GJB1	gap junction protein, beta 1, 32kD
181	204995_at	AL567411		cyclin-dependent kinase 5, regulatory sub 1 (p35)
182	205051_s_at	NM_000222	KIT	v-kit Hardy-Zuckerman 4 feline

				sarcoma viral oncogene homolog
183	205142_x_at	NM_000033	ABCD1	ATP-binding cassette, sub-family D (ALD), mem 1
184	205169_at	NM_005057	RBBP5	retinoblastoma-binding protein 5
185	205373_at	NM_004389	CTNNA2	catenin alpha 2
186	205376_at	NM_003866	INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kD
187	205405_at	NM_003966	SEMA5A	sema domain, seven thrombospondin repeats, transmembrane domain and short cytoplasmic domain 5A
188	205447_s_at	BE222201		mitogen-activated protein kinase kinase kinase 12
189	205458_at	BG034972		melanocortin 1 receptor
190	205566_at	NM_007011	HS1-2	putative transmembrane protein
191	205591_at	NM_006334	AMY	neuroblastoma (nerve tissue) protein
192	205681_at	NM_004049	BCL2A1	BCL2-related protein A1
193	205690_s_at	NM_003910	G10	maternal G10 transcript
194	205691_at	NM_004209	SYNGR3	synaptogyrin 3
195	205717_x_at	NM_002588	PCDHGC3	protocadherin gamma subfamily C, 3
196	205813_s_at	NM_000429	MAT1A	methionine adenosyltransferase I, α
197	205937_at	NM_006569	CGR11	cell growth regulatory with EF-hand domain
198	205945_at	NM_000565	IL6R	interleukin 6 receptor
199	205996_s_at	NM_013411	AK2 B	adenylate kinase 2
200	206128_at	AI264306		adrenergic, alpha-2C-, receptor
201	206307_s_at	NM_004472	FOXD1	forkhead box D1
202	206332_s_at	NM_005531	IFI16	interferon, gamma-inducible 16
203	206397_x_at	NM_001492	GDF1	growth differentiation factor 1
204	206441_s_at	NM_017828	FLJ20452	hypothetical protein FLJ20452
205	206462_s_at	NM_002530	NTRK3	neurotrophic tyrosine kinase, receptor, type 3
206	206503_x_at	NM_002675	PML	promyelocytic leukemia
207	206617_s_at	NM_002910	RENBP	renin-binding protein
208	206630_at	NM_000372	TYR	tyrosinase
209	206688_s_at	NM_006693	CPSF4	cleavage and polyadenylation specific factor 4, 30kD subunit
210	206696_at	NM_000273	OA1	ocular albinism 1
211	206777_s_at	NM_000496	CRYBB2	crystallin, beta B2
212	206864_s_at	NM_003806	HRK	harakiri, BCL2-interacting protein
213	206976_s_at	NM_006644	HSP105B	heat shock 105kD
214	207038_at	NM_004694	SLC16A6	solute carrier family 16 member 6
215	207060_at	NM_001427	EN2	engrailed homolog 2
216	207144_s_at	NM_004143	CITED1	Cbpp300-interacting transactivator, with GluAsp-rich carboxy-terminal domain, 1
217	207163_s_at	NM_005163	AKT1	v-akt murine thymoma viral oncogene homolog 1
218	207183_at	NM_006143	GPR19	G protein-coupled receptor 19
219	207592_s_at	NM_001194	HCN2	hyperpolarization activated cyclic nucleotide-gated potassium channel 2
220	207614_s_at	NM_003592	CUL1	cullin 1
221	207622_s_at	NM_005692	ABCF2	ATP-binding cassette, sub-fam F mem 2
222	207828_s_at	NM_005196	CENPF	centromere protein F

223	208002_s_at	NM_007274	HBACH	cytosolic acyl coenzyme A thioester hydrolase
224	208089_s_at	NM_030794	FLJ21007	hypothetical protein FLJ21007
225	208308_s_at	NM_000175	GPI	glucose phosphate isomerase
226	208540_x_at	NM_021039	S100A14	S100 calcium-binding protein A14
227	208644_at	M32721		poly(ADP-ribose) polymerase
228	208657_s_at	AF142408		cell division control protein septin D1
229	208677_s_at	AL550657		Basigin
230	208696_at	AF275798		PNAS-102
231	208710_s_at	AI424923		adaptor-related protein complex 3, delta 1 subunit
232	208723_at	BC000350		ubiquitin specific protease 11
233	208744_x_at	BG403660		heat shock 105kD
234	208837_at	BC000027		integral type I protein
235	208916_at	AF105230	SLC1A5	neutral amino acid transporter
236	208928_at	AF258341		NADPH-cytochrome P450 reductase
237	208956_x_at	U62891	DUT	deoxyuridine triphosphatase
238	208974_x_at	BC003572		karyopherin (importin) beta 1
239	208975_s_at	L38951		importin beta subunit
240	209015_s_at	BC002446		MRJ gene for a member of DNAJ fam
241	209036_s_at	BC001917		malate dehydrogenase 2, NAD
242	209053_s_at	AF083389		Wolf-Hirschhorn syn candidate 1
243	209072_at	M13577	MBP	myelin basic protein
244	209079_x_at	AF152318	PCDH-gamma-A1	protocadherin gamma A1
245	209081_s_at	NM_030582	COL18A1	collagen, type XVIII, alpha 1
246	209123_at	BC000576		quinoid dihydropteridine reductase
247	209132_s_at	BE313890		hypothetical protein FLJ20452
248	209172_s_at	U30872		mitosin
249	209197_at	AA626780		KIAA0080 protein
250	209198_s_at	BC004291		Similar to synaptotagmin 11
251	209247_s_at	BC001661		ATP-binding cassette, sub-fam F mem 2
252	209254_at	AF277177		KIAA0265 protein
253	209255_at	AF277177		KIAA0265 protein
254	209256_s_at	AF277177		PNAS-119
255	209283_at	AF007162		unknown mRNA
256	209345_s_at	AL561930		phosphatidylinositol 4-kinase type II
257	209407_s_at	AF068892		Dukes type B colon adenocarcinoma truncated suppressin
258	209515_s_at	U38654		Rab27a
259	209773_s_at	BC001886		ribonucleotide reductase M2 polypep
260	209825_s_at	BC002906		Sim to uridine monophosphate kinase
261	209827_s_at	NM_004513	IL16	interleukin 16
262	209828_s_at	M90391		putative IL-16 protein precursor
263	209848_s_at	U01874		me20m
264	209875_s_at	M83248		nephropontin
265	209932_s_at	U90223		deoxyuridine triphosphate nucleotidohydrolase precursor
266	210052_s_at	AF098158		restricted expressed proliferation associated protein 100
267	210073_at	L32867		alpha 2,8-sialyltransferase
268	210111_s_at	AF277175		PNAS-138
269	210127_at	BC002510		small GTPase RAB6B

270	210854_x_at	U17986		GABAnoradrenaline transporter
271	210926_at	AY014272	FKSG30	FKSG30
272	210948_s_at	AF294627	LEF1	lymphoid enhancer factor 1 isoform
273	210951_x_at	AF125393		Rab27 isoform
274	211013_x_at	AF230411		tripartite motif protein TRIM19 lambda
275	211052_s_at	BC006364		clone MGC:12705,
276	211066_x_at	BC006439		Similar to protocadherin gamma subfamily A, 5
277	211373_s_at	U34349	AD3LPAD5	seven trans-membrane domain
278	211564_s_at	BC003096		Sim to LIM domain protein
279	211752_s_at	BC005954		clone MGC:14592
280	211759_x_at	BC005969		clone MGC:14625
281	211833_s_at	U19599		BAX delta
282	212000_at	AB002363		KIAA0365 gene product
283	212070_at	AL554008		G protein-coupled receptor 56
284	212081_x_at	AF129756	MSH55	MSH55
285	212119_at	BF348067		phosphatidylinositol glycan, class F
286	212178_s_at	AK022555		FLJ12493 fis
287	212193_s_at	BE881529		KIAA0731 protein
288	212247_at	AW008531		KIAA0225 protein
289	212285_s_at	AF016903		IMAGE:3506210
290	212312_at	AL117381		clone RP5-857M17 on chrom 20
291	212338_at	AA621962		KIAA0727 protein
292	212402_at	BE895685		KIAA0853 protein
293	212472_at	BE965029		FLJ22463 fis
294	212473_s_at	BE965029		FLJ22463 fis
295	212512_s_at	AA551784		coactivator-associated arginine methyltransferase-1
296	212520_s_at	AI684141		SWISNF related
297	212552_at	BE617588		hippocalcin-like
298	212715_s_at	AB020626		KIAA0819 protein
299	212739_s_at	AL523860		non-metastatic cells 4
300	212744_at	AI813772		clone HQ0692
301	212745_s_at	AI813772		clone HQ0692
302	212793_at	BF513244		KIAA0381 protein
303	212796_s_at	BF195608		KIAA1055 protein
304	213002_at	BF347326	MARCKS, 80K-L	myristoylated alanine-rich protein kinase C substrate
305	213007_at	BG478677		polymerase (DNA directed), gamma
306	213008_at	BG478677		polymerase (DNA directed), gamma
307	213096_at	T51252		KIAA0481 gene product
308	213131_at	R38389		olfactomedin related ER localized
309	213169_at	BG109855		clone TUA8 Cri-du-chat region
310	213215_at	AI910895		clone EUROIMAGE 42138
311	213217_at	AU149572		adenylate cyclase 2(brain)
312	213241_at	AF035307		clone 23785
313	213274_s_at	BE875786		cathepsin B
314	213275_x_at	BE875786		cathepsin B
315	213330_s_at	BE886580		stress-induced-phosphoprotein 1
316	213333_at	AL520774		malate dehydrogenase 2, NAD
317	213338_at	BF062629		DKFZP586E1621 protein
318	213392_at	AW070229		G protein-coupled receptor, fam C, group 5, mem B
319	213474_at	AI890903		ESTs

320	213496_at	AW592563		KIAA0455 gene product
321	213573_at	AA861608		karyopherin (importin) beta 1
322	213587_s_at	AI884867		ribosomal protein L26
323	213638_at	AW054711		PAC 257A7 on chromosome 6p24
324	213670_x_at	AI768378		KIAA0618 gene product
325	213720_s_at	AI831675		SWISNF related, matrix associated, actin dependent regulator of chromatin, subfam a, member 4
326	213746_s_at	AW051856		filamin A, alpha
327	213836_s_at	AW052084		KIAA1001 protein
328	213895_at	BF445047		epithelial membrane protein 1
329	213960_at	T87225		CLONE=IMAGE:22392
330	214023_x_at	AL533838		tubulin, beta polypeptide
331	214068_at	AF070610		clone 24505
332	214104_at	AI703188		G-protein coupled receptor
333	214201_x_at	AA742237		HLA-B associated transcript-2
334	214581_x_at	BE568134		death receptor 6
335	214614_at	AI738662		homeo box HB9
336	214632_at	AA295257		neuropilin 2
337	214656_x_at	BE790157		myosin IB
338	214687_x_at	AK026577		FLJ22924 fis
339	214708_at	BG484314		syntrophin, beta 1
340	214710_s_at	BE407516		cyclin B1
341	214714_at	AK022360		FLJ12298 fis
342	214717_at	AL137534		DKFZp434H1419
343	214752_x_at	AI625550		filamin A, alpha
344	214778_at	AB011541		MEGF8
345	214841_at	AF070524		clone 24453
346	214893_x_at	AI421964		hyperpolarization activated cyclic nucleotide-gated potassium channel 2
347	214896_at	AL109671		EUROIMAGE 29222
348	215025_at	S76476		trkC {alternatively spliced}
349	215115_x_at	AI613045		ets variant gene 6 (TEL oncogene)
350	215126_at	AL109716		EUROIMAGE 208948
351	215155_at	J04178	HEXA	abnormal β-hexosaminidase α chain
352	215311_at	AL109696		EUROIMAGE 21920
353	215812_s_at	U41163	SLC6A10	creatine transporter
354	215836_s_at	AK026188		FLJ22535 fis
355	216194_s_at	AD001527		DNA from chrom 19-cosmid f24590 containing CAPNS and POL2RI
356	216973_s_at	S49765		homeo box B7
357	217033_x_at	S76475	trkC	neurotrophic tyrosine kinase, receptor, type 3
358	217104_at	AL109714		EUROIMAGE 327506
359	217226_s_at	M95929	PHOX1	Paired mesoderm homeo box 1
360	217297_s_at	AF143684	MYO9b	unconventional myosin IXb
361	217377_x_at	AF041811	ETV6-NTRK3 fusion	ETS related protein-growth factor receptor tyrosine kinase fusion proteins
362	217419_x_at	AK021586		FLJ11524 fis
363	217624_at	AA464753		ESTs
364	217799_x_at	NM_003344	UBE2H	ubiquitin-conjugating enzyme E2H
365	217827_s_at	NM_016630	ACP33	acid cluster protein 33
366	217867_x_at	NM_012105	BACE2	beta-site APP-cleaving enzyme 2

367	217874_at	NM_003849	SUCLG1	succinate-CoA ligase, GDP-forming, alpha subunit
368	217891_at	NM_022744	FLJ13868	hypothetical protein FLJ13868
369	218009_s_at	NM_003981	PRC1	protein regulator of cytokinesis 1
370	218030_at	NM_014030	GIT1	G protein-coupled receptor kinase-interactor 1
371	218074_at	NM_016062	LOC51647	CGI-128 protein
372	218143_s_at	NM_005697	SCAMP2	secretory carrier membrane protein 2
373	218151_x_at	NM_024531	FLJ11856	hypothetical protein FLJ11856
374	218152_at	NM_018200	HMG20A	high-mobility group 20A
375	218161_s_at	NM_017882	FLJ20561	hypothetical protein FLJ20561
376	218175_at	NM_025140	FLJ22471	hypothetical protein FLJ22471
377	218330_s_at	NM_018162	FLJ10633	hypothetical protein FLJ10633
378	218349_s_at	NM_017975	FLJ10036	hypothetical protein FLJ10036
379	218359_at	NM_024958	FLJ23329	hypothetical protein FLJ23329
380	218376_s_at	NM_022765	FLJ11937	hypothetical protein FLJ11937
381	218447_at	NM_020188	DC13	DC13 protein
382	218542_at	NM_018131	FLJ10540	hypothetical protein FLJ10540
383	218564_at	BC002574	FLJ10520	hypothetical protein FLJ10520
384	218618_s_at	NM_022763	FLJ23399	hypothetical protein FLJ23399
385	218678_at	NM_024609	FLJ21841	hypothetical protein FLJ21841
386	218774_at	NM_014026	HSPC015	HSPC015 protein
387	218786_at	NM_016575	TU12B1-TY	TU12B1-TY protein
388	218824_at	NM_018215	FLJ10781	hypothetical protein FLJ10781
389	218839_at	NM_012258	HEY1	hairyenhancer-of-split related with YRPW motif 1
390	218856_at	NM_016629	LOC51323	hypothetical protein LOC51323
391	218888_s_at	NM_018092	FLJ10430	hypothetical protein FLJ10430
392	218931_at	NM_022449	FLJ12538	hypothetical protein FLJ12538
393	218952_at	NM_013271	SAAS	granin-like neuroendocrine peptide precursor
394	218956_s_at	NM_015545	KIAA0632	KIAA0632 protein
395	218980_at	NM_025135	KIAA1695	hypothetical protein FLJ22297
396	218996_at	NM_013342	TFPT	TCF3 (E2A) fusion partner
397	219011_at	NM_020904	PLEKHA4	pleckstrin homology domain-containing, family A member 4
398	219039_at	NM_017789	FLJ20369	hypothetical protein FLJ20369
399	219040_at	NM_024535	FLJ22021	hypothetical protein FLJ22021
400	219041_s_at	NM_014374	AP4	zinc finger protein
401	219051_x_at	NM_024042	MGC2601	hypothetical protein MGC2601
402	219066_at	NM_021823	MDS018	hypothetical protein MDS018
403	219066_at	NM_021823	MDS018	hypothetical protein MDS018
404	219148_at	NM_018492	TOPK	PDZ-binding kinase; T-cell originated protein kinase
405	219152_at	NM_015720	PODLX2	endoglycan
406	219219_at	NM_017854	FLJ20512	hypothetical protein FLJ20512
407	219361_s_at	NM_022767	FLJ12484	hypothetical protein FLJ12484
408	219372_at	NM_014055	CDV-1	CDV-1 protein
409	219408_at	NM_019023	FLJ10640	hypothetical protein FLJ10640
410	219478_at	NM_021197	WFDC1	WAP four-disulfide core domain 1
411	219491_at	NM_024036	MGC3103	hypothetical protein MGC3103
412	219522_at	NM_014344	FJX1	putative secreted ligand homologous to fjx1
413	219537_x_at	NM_016941	DLL3	Delta (Drosophila)-like 3

414	219555_s_at	NM_018455	BM039	uncharacterized bone marrow protein BM039
415	219578_s_at	NM_030594	FLJ13203	hypothetical protein FLJ13203
416	219634_at	NM_018413	C4ST	chondroitin 4-sulfotransferase
417	219637_at	NM_025139	FLJ12584	hypothetical protein FLJ12584
418	219703_at	NM_018365	FLJ11222	hypothetical protein FLJ11222
419	219742_at	NM_030567	MGC10772	hypothetical protein MGC10772
420	219895_at	NM_017938	FLJ20716	hypothetical protein FLJ20716
421	219933_at	NM_016066	LOC51022	CGI-133 protein
422	220116_at	NM_021614	KCNN2	potassium intermediatesmall conductance calcium-activated channel, subfamily N, member 2
423	220155_s_at	NM_023924	FLJ13441	hypothetical protein FLJ13441
424	220178_at	NM_021731	PP3501	hypothetical protein PP3501
425	220454_s_at	NM_020796	SEMA6A	sema domain, transmembrane domain and cytoplasmic domain, 6A
426	220864_s_at	NM_015965	LOC51079	CGI-39 protein; cell death-regulatory protein GRIM19
427	220948_s_at	NM_000701	ATP1A1	ATPase, Na+K+ transporting, alpha 1 polypeptide
428	220973_s_at	NM_030974		hypothetical protein DKFZp434N1923
429	220974_x_at	NM_030971	BA108L7.2	similar to rat tricarboxylate carrier-like
430	220980_s_at	NM_031284		hypothetical protein DKFZp434B195
431	221059_s_at	NM_021615	CHST6	carbohydrate sulfotransferase 6
432	221483_s_at	AF084555	ARPP-19	okadaic acid-inducible and cAMP-regulated phosphoprotein 19
433	221484_at	NM_004776		UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5
434	221538_s_at	AL136663		DKFZp564A176
435	221558_s_at	AF288571	LEF1	lymphoid enhancer factor-1
436	221577_x_at	AF003934		prostate differentiation factor
437	221641_s_at	AF241787		CGI16-iso
438	221688_s_at	AL136913		DKFZp586L0118
439	221710_x_at	BC006241		hypothetical protein FLJ10647
440	221732_at	AK026161		FLJ22508 fis
441	221759_at	AL583123		CLONE=CS0DL009YN09
442	221797_at	AY007126		clone CDABP0028
443	221799_at	AB037823		KIAA1402 protein,
444	221815_at	BE671816		ESTs
445	221882_s_at	AI636233		five-span transmembrane protein M83
446	221902_at	AL567940		CLONE=CS0DF036YK19
447	221909_at	BF984207		ESTs
448	221962_s_at	AI829920		ubiquitin-conjugating enzyme E2H
449	222116_s_at	AL157485		DKFZp762O207
450	222153_at	AK023133		FLJ13071 fis
451	222155_s_at	AK021918		FLJ11856 fis
452	222175_s_at	AK000003		FLJ00003 protein
453	222196_at	AK000470		FLJ20463 fis
454	222199_s_at	AK001289		FLJ10427 fis
455	222206_s_at	AA781143		EUROIMAGE 2021883
456	222212_s_at	AK001105		FLJ10243 fis
457	222231_s_at	AK025328		FLJ21675 fis
458	222234_s_at	AK022644		FLJ12582 fis
459	222240_s_at	AL137749		DKFZp434A0612

460	222294_s_at	AW971415		ESTs
461	32811_at	X98507		myosin-I beta
462	40560_at	U28049	TXB2	TBX2
463	44783_s_at	R61374		IMAGE-37665
464	46665_at	AI949392		IMAGE-2470926
465	55093_at	AA534198		IMAGE-993116
466	63825_at	AI557319		
467	87100_at	AI832249		
468	200017_at	NM_002954	RPS27A	ribosomal protein S27a
469	200606_at	NM_004415	DSP	desmoplakin (DPI, DPII)
470	200632_s_at	NM_006096	NDRG1	N-myc downstream regulated
471	200636_s_at	NM_002840	PTPRF	protein tyrosine phosphatase, receptor type, F
472	200795_at	NM_004684	SPARCL1	SPARC-like 1
473	200810_s_at	NM_001280	CIRBP	cold inducible RNA-binding protein
474	200897_s_at	NM_016081	KIAA0992	Palladin
475	200953_s_at	NM_001759	CCND2	cyclin D2
476	200965_s_at	NM_006720	ABLIM-s	actin binding LIM protein 1 transcript variant
477	201012_at	NM_000700	ANXA1	annexin A1
478	201041_s_at	NM_004417	DUSP1	dual specificity phosphatase 1
479	201125_s_at	NM_002213	ITGB5	integrin, beta 5
480	201200_at	NM_003851	CREG	cellular repressor of E1A-stimulated genes
481	201286_at	Z48199	syndecan 1	syndecan-1 gene (exons 2-5)
482	201328_at	AL575509		v-ets avian erythroblastosis virus E26 oncogene homolog 2
483	201425_at	NM_000690	ALDH2	aldehyde dehydrogenase 2, mitochondrial
484	201427_s_at	NM_005410	SEPP1	selenoprotein P, plasma, 1
485	201432_at	NM_001752	CAT	Catalase
486	201540_at	NM_001449	FHL1	four and a half LIM domains 1
487	201667_at	NM_000165	GJA1	gap junction protein, alpha 1, 43kD
488	201681_s_at	AB011155	KIAA0583	KIAA0583
489	201798_s_at	NM_013451	FER1L3	fer-1 (C.elegans)-like 3 (myoferlin)
490	201820_at	NM_000424	KRT5	keratin 5
491	201829_at	AW263232	NET1	neuroepithelial cell transf gene 1
492	201830_s_at	NM_005863	NET1	neuroepithelial cell transf gene 1
493	201839_s_at	NM_002354	TACSTD1	tumor-associated calcium signal transducer 1
494	201842_s_at	AI826799		EGF-containing fibulin-like extracellular matrix protein 1
495	201843_s_at	NM_004105	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1 transcript variant 1
496	201983_s_at	AW157070		epidermal growth factor receptor
497	201984_s_at	NM_005228	EGFR	epidermal growth factor receptor
498	202054_s_at	NM_000382	ALDH3A2	aldehyde dehydrogenase 3 family, member A2
499	202085_at	NM_004817	TJP2	tight junction protein 2
500	202193_s_at	NM_001144	AMFR	autocrine motility factor receptor
501	202196_s_at	NM_013253	DKK3	dickkopf (Xenopus laevis) homolog 3
502	202242_at	NM_004615	TM4SF2	transmembrane 4 superfamily mem 2
503	202267_at	NM_005562	LAMC2	laminin, gamma 2, transcript variant 1

504	202286_s_at	J04152	GA733-1	gastrointestinal tumor-assoc antigen
505	202289_s_at	NM_006997	TACC2	transforming, acidic coiled-coil containing protein 2
506	202350_s_at	NM_002380	MATN2	matrilin 2 precursor, transcript var 1
507	202387_at	NM_004323	BAG1	BCL2-associated athanogene
508	202489_s_at	BC005238		FXYD domain-containing ion transport regulator 3
509	202525_at	NM_002773	PRSS8	protease, serine, 8 (prostasin)
510	202552_s_at	NM_016441	CRIM1	cysteine-rich motor neuron 1
511	202565_s_at	NM_003174	SVIL	supervillin transcript variant 1
512	202575_at	NM_001878	CRABP2	cellular retinoic acid-binding protein 2
513	202597_at	AU144284		interferon regulatory factor 6
514	202668_at	BF001670		ephrin-B2
515	202712_s_at	NM_020990	CKMT1	creatine kinase, mitochondrial 1 nuclear gene mitochondrial protein
516	202746_at	AL021786		PAC 696H22 on chrom Xq21.1-21.2
517	202826_at	NM_003710	SPINT1	serine protease inhibitor, Kunitz t 1
518	202890_at	T62571		microtubule-associated protein 7
519	202936_s_at	NM_000346	SOX9	SRY-box 9
520	202994_s_at	Z95331		clone CTA-941F9 on chrom 22q13
521	203037_s_at	NM_014751	KIAA0429	KIAA0429 gene product
522	203074_at	NM_001630	ANXA8	annexin A8
523	203081_at	NM_020248	LOC56998	beta-catenin-interacting protein ICAT
524	203126_at	NM_014214	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2
525	203178_at	NM_001482	GATM	glycine amidinotransferase
526	203240_at	NM_003890	FC(γ)BP	IgG Fc binding protein
527	203327_at	N22903		insulin-degrading enzyme
528	203355_s_at	NM_015310	KIAA0942	KIAA0942 protein
529	203407_at	NM_002705	PPL	Periplakin
530	203408_s_at	NM_002971	SATB1	special AT-rich sequence binding protein 1
531	203430_at	NM_014320	SOUL	putative heme-binding protein
532	203453_at	NM_001038	SCNN1A	Na channel, nonvoltage-gated 1 α
533	203485_at	NM_021136	RTN1	reticulon 1
534	203549_s_at	NM_000237	LPL	lipoprotein lipase
535	203571_s_at	NM_006829	APM2	adipose specific 2
536	203585_at	NM_007150	ZNF185	zinc finger protein 185 (LIM domain)
537	203636_at	BE967532	MID1	midline 1 (OpitzBBB syndrome)
538	203637_s_at	NM_000381	MID1	
539	203638_s_at	NM_022969	FGFR2	FGF receptor 2 transcript var 2
540	203678_at	NM_014967	KIAA1018	KIAA1018 protein
541	203687_at	NM_002996	SCYD1	small inducible cytokine subfam D (Cys-X3-Cys) mem 1
542	203726_s_at	NM_000227	LAMA3	laminin, alpha 3
543	203786_s_at	NM_003287	TPD52L1	tumor protein D52-like 1
544	203797_at	AF039555	VSNL1	visinin-like protein 1
545	203799_at	NM_014880	KIAA0022	KIAA0022 gene product
546	203812_at	AB011538		MEGF5
547	203881_s_at	NM_004010		dystrophin transcript variant Dp427p2
548	203910_at	NM_004815	PARG1	PTPL1-associated RhoGAP 1
549	203917_at	NM_001338	CXADR	coxsackie virus and adenovirus receptor
550	203961_at	AL157398	NEBL	nebulette protein

551	203962_s_at	NM_006393	NEBL	
552	203963_at	NM_001218	CA12	carbonic anhydrase XII
553	203992_s_at	AF000992	UTX	ubiquitous TPR motif, X isoform alternative transcript 1
554	203997_at	NM_002829	PTPN3	protein tyrosine phosphatase, non-receptor type 3
555	204005_s_at	NM_002583	PAWR	PRKC, apoptosis, WT1, regulator
556	204019_s_at	NM_015677		hypothetical protein DKFZP586F1318
557	204036_at	AW269335		endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2
558	204037_at	AW269335		endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2
559	204042_at	AB020707		KIAA0900 protein
560	204058_at	AL049699		clone 747H23 on chrom 6q13-15
561	204059_s_at	NM_002395	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic
562	204072_s_at	NM_023037	13CDNA73	putative gene product
563	204112_s_at	NM_006895	HNMT	histamine N-methyltransferase
564	204135_at	NM_014890	DOC1	downregulated in ovarian cancer 1
565	204136_at	NM_000094	COL7A1	collagen, type VII, alpha 1
566	204151_x_at	NM_001353	AKR1C1	aldo-keto reductase family 1, mem C1
567	204154_at	NM_001801	CDO1	cysteine dioxygenase, type I
568	204168_at	NM_002413	MGST2	microsomal glutathione S-transferase 2
569	204201_s_at	NM_006264	PTPN13	protein tyrosine phosphatase, non-receptor type 13
570	204204_at	NM_001860	SLC31A2	solute carrier family 31 member 2
571	204224_s_at	NM_000161	GCH1	GTP cyclohydrolase 1
572	204254_s_at	NM_000376	VDR	vitamin D receptor
573	204345_at	NM_001856	COL16A1	collagen, type XVI, alpha 1
574	204351_at	NM_005980	S100P	S100 calcium-binding protein P
575	204359_at	NM_013231	FLRT2	fibronectin leucine rich transmembrane protein 2
576	204363_at	NM_001993	F3	coagulation factor III
577	204379_s_at	NM_000142	FGFR3	fibroblast growth factor receptor 3
578	204388_s_at	NM_000240	MAOA	monoamine oxidase A
579	204389_at	NM_000240	MAOA	monoamine oxidase A
580	204400_at	NM_005864	EFS2	signal transduction protein
581	204421_s_at	M27968	FGF	basic fibroblast growth factor
582	204422_s_at	NM_002006	FGF2	fibroblast growth factor 2 (basic)
583	204424_s_at	AL050152		neuronal specific transcription factor DAT1
584	204455_at	NM_001723	BPAG1	bullous pemphigoid antigen 1
585	204503_at	NM_001988	EVPL	envoplakin
586	204517_at	BE962749	cyclophilin C	peptidylprolyl isomerase C
587	204519_s_at	NM_015993	LOC51090	plasmolipin
588	204537_s_at	NM_004961	GABRE	gamma-aminobutyric acid A receptor, epsilon transcript variant 1
589	204591_at	NM_006614	CHL1	cell adhesion molecule with homology to L1CAM
590	204600_at	NM_004443	EPHB3	EphB3
591	204671_s_at	BE677131		KIAA0957 protein
592	204675_at	NM_001047	SRD5A1	steroid-5-α-reductase, α polypeptide 1

593	204718_at	NM_004445	EPHB6	EphB6
594	204719_at	NM_007168	ABCA8	ATP-binding cassette, sub-fam A mem 8
595	204734_at	NM_002275	KRT15	keratin 15
596	204749_at	NM_004538	NAP1L3	nucleosome assembly protein 1-like 3
597	204753_s_at	AI810712		hepatic leukemia factor
598	204754_at	AI810712		hepatic leukemia factor
599	204755_x_at	M95585	HLF	leukemia factor
600	204765_at	NM_005435	ARHGEF5	Rho guanine nucleotide exchange factor 5
601	204773_at	NM_004512	IL11RA	interleukin 11 receptor, alpha
602	204773_at	NM_004512	IL11RA	
603	204855_at	NM_002639	SERPINB5	serine (or cysteine) proteinase inhibitor, clade B, member 5
604	204872_at	NM_007005	BCE-1	BCE-1 protein
605	204937_s_at	NM_016325	ZNF274	zinc finger protein 274
606	204942_s_at	NM_000695	ALDH3B2	aldehyde dehydrogenase 3 fam mem B2
607	204952_at	NM_014400	C4.4A	GPI-anchored metastasis-associated protein homolog
608	204971_at	NM_005213	CSTA	cystatin A (stefin A)
609	204975_at	NM_001424	EMP2	epithelial membrane protein 2
610	204990_s_at	NM_000213	ITGB4	integrin, beta 4
611	205014_at	NM_005130	HBP17	heparin-binding growth factor binding
612	205019_s_at	NM_004624	VIPR1	vasoactive intestinal pep receptor 1
613	205081_at	NM_001311	CRIP1	cysteine-rich protein 1 (intestinal)
614	205109_s_at	NM_015320	ARHGEF4	Rho guanine nucleotide exchange factor (GEF) 4
615	205128_x_at	NM_000962	PTGS1	prostaglandin-endoperoxide synthase 1
616	205185_at	NM_006846	SPINK5	serine protease inhibitor, Kazal t, 5
617	205200_at	NM_003278	TNA	tetranectin
618	205206_at	NM_000216	KAL1	Kallmann syndrome 1 sequence
619	205236_x_at	NM_003102	SOD3	superoxide dismutase 3, extracellular
620	205251_at	NM_022817	PER2	period homolog 2 transcript variant 1
621	205259_at	NM_000901	NR3C2	nuclear receptor subfamily 3, group C, member 2
622	205286_at	U85658		transcription factor ERF-1
623	205349_at	NM_002068	GNA15	guanine nucleotide binding protein, α 15
624	205363_at	NM_003986	BBOX1	butyrobetaine (γ), 2-oxoglutarate dioxygenase 1
625	205382_s_at	NM_001928	DF	D component of complement (adipsin)
626	205384_at	NM_005031	FXYD1	FXYD domain-containing ion transport regulator 1 variant a
627	205403_at	NM_004633	IL1R2	interleukin 1 receptor, type II
628	205404_at	NM_005525	HSD11B1	hydroxysteroid dehydrogenase 1
629	205407_at	NM_021111	RECK	reversion-inducing-cysteine-rich protein with kazal motifs
630	205440_s_at	NM_000909	NPY1R	neuropeptide Y receptor Y1
631	205455_at	NM_002447	MST1R	macrophage stimulating 1 receptor
632	205464_at	NM_000336	SCNN1B	Na channel, nonvoltage-gated 1, β
633	205470_s_at	NM_006853	KLK11	kallikrein 11
634	205490_x_at	BF060667	connexin 31	gap junction protein, beta 3, 31kD
635	205498_at	NM_000163	GHR	growth hormone receptor
636	205559_s_at	NM_006200	PCSK5	proprotein convertase subtilisinkexin

				type 5
637	205560_at	NM_006200	PCSK5	
638	205569_at	NM_014398	TSC403	similar to lysosome-associated membrane glycoprotein
639	205613_at	NM_016524	LOC51760	BK protein
640	205668_at	NM_002349	LY75	lymphocyte antigen 75
641	205672_at	NM_000380	XPA	xeroderma pigmentosum, complementation group A
642	205709_s_at	NM_001263	CDS1	CDP-diacylglycerol synthase 1
643	205730_s_at	NM_014945	KIAA0843	KIAA0843 protein
644	205765_at	NM_000777	CYP3A5	cyt P450, subfam IIIA, polypep 5
645	205807_s_at	NM_020127	TUFT1	tuftelin 1
646	205857_at	AI269290		solute carrier family 18, member 2
647	205883_at	NM_006006	ZNF145	zinc finger protein 145
648	205900_at	NM_006121	KRT1	keratin 1
649	205933_at	NM_015559	KIAA0437	KIAA0437 protein
650	205977_s_at	NM_005232	EPHA1	EphA1
651	206032_at	AI797281		est:we86g02.x1
652	206033_s_at	NM_001941	DSC3	desmocollin 3 transcript variant Dsc3a
653	206068_s_at	AI367275		acyl-Coenzyme A dehydrogenase, long chain
654	206093_x_at	NM_007116	TNXA	tenascin XA
655	206122_at	NM_006942	SOX20	SRY-box 20
656	206149_at	NM_022097	LOC63928	hepatocellular carcinoma antigen gene 520
657	206170_at	NM_000024	ADRB2	adrenergic, beta-2-, receptor, surface
658	206192_at	L20815		S protein
659	206201_s_at	NM_005924	MEOX2	mesenchyme homeo box 2
660	206276_at	NM_003695	E48	lymphocyte antigen 6 comp locus D
661	206315_at	NM_004750	CRLF1	cytokine receptor-like factor 1
662	206363_at	NM_005360	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog
663	206385_s_at	NM_020987	ANK3	ankyrin 3, node of Ranvier, tran var 1
664	206400_at	NM_002307	LGALS7	lectin, galactoside-binding, soluble, 7
665	206453_s_at	NM_016250	NDRG2	N-myc downstream-regulated gene 2
666	206481_s_at	NM_001290	LDB2	LIM domain binding 2
667	206482_at	NM_005975	PTK6	PTK6 protein tyrosine kinase 6
668	206515_at	NM_000896	CYP4F3	cyt P450, subfam IVF, polypeptide 3
669	206539_s_at	NM_023944	CYP4F12	cytochrome P450 isoform 4F12
670	206581_at	NM_001717	BNC	basonuclin
671	206637_at	NM_014879	KIAA0001	KIAA0001 gene product
672	206655_s_at	NM_000407	GP1BB	glycoprotein Ib (platelet), β polypep
673	206693_at	NM_000880	IL7	interleukin 7
674	206884_s_at	NM_003843	SCEL	sciellin
675	207002_s_at	NM_002656	PLAGL1	pleiomorphic adenoma gene-like 1
676	207023_x_at	NM_000421	KRT10	keratin 10
677	207076_s_at	NM_000050	ASS	argininosuccinate synthetase
678	207121_s_at	NM_002748	MAPK6	mitogen-activated protein kinase 6
679	207655_s_at	NM_013314	SLP65	B cell linker protein
680	207720_at	NM_000427	LOR	loricrin
681	207761_s_at	NM_014033		DKFZP586A0522 protein
682	207843_x_at	NM_001914	CYB5	cytochrome b-5
683	207908_at	NM_000423	KRT2A	keratin 2A
684	207943_x_at	NM_006718	PLAGL1	pleiomorphic adenoma gene-like transcript variant 2

685	207955_at	NM_006664	SCYA27	small inducible cytokine subfamily A (Cys-Cys), member 27
686	207996_s_at	NM_004338	C18ORF1	chrom 18 open reading frame 1
687	208096_s_at	NM_030820		hypothetical protein DKFZp564B052
688	208146_s_at	NM_031311	LOC54504	serine carboxypeptidase vitellogenic-like
689	208161_s_at	NM_020037	ABCC3	ATP-binding cassette sub-fam C mem 3
690	208190_s_at	NM_015925	LISCH7	liver-specific bHLH-Zip transcription factor
691	208228_s_at	M87771	K-sam-III	secreted FGF receptor
692	208609_s_at	NM_019105	TNXB	tenascin XB
693	208614_s_at	M62994		thyroid autoantigen
694	208651_x_at	M58664		CD24 signal transducer
695	208690_s_at	BC000915		Similar to LIM protein,
696	208798_x_at	AF204231	GM88	88-kDa Golgi protein
697	209047_at	AL518391		aquaporin 1
698	209159_s_at	AV724216		NDRG family, member 4
699	209160_at	AB018580	hluPGFS	aldo-keto reductase family 1, mem C3
700	209211_at	AF132818	CKLF	colon Kruppel-like factor
701	209212_s_at	AB030824		transcription factor BTEB2
702	209289_at	AI700518		nuclear factor IB
703	209290_s_at	BC001283		Similar to nuclear factor IB,
704	209309_at	D90427		zinc-alpha2-glycoprotein
705	209318_x_at	BG547855		pleiomorphic adenoma gene-like 1
706	209335_at	AI281593		decorin
707	209348_s_at	AF055376	c-maf	short form transcription factor C-MAF
708	209351_at	BC002690		keratin 14
709	209357_at	AF109161	MRG1	p35srj
710	209366_x_at	M22865		cytochrome b5
711	209368_at	AF233336	EPHX2	soluble epoxide hydrolase
712	209386_at	AI346835		transmembrane 4 superfam mem 1
713	209392_at	L35594	autotaxin	ectonucleotide pyrophosphatasephosphodiesterase 2
714	209465_x_at	AL565812		pleiotrophin
715	209493_at	AF338650	AIPC	PDZ domain-containing protein AIPC
716	209540_at	NM_000618	somatomedin C	insulin-like growth factor 1
717	209550_at	U35139		NECDIN related protein
718	209558_s_at	AB013384	HIP1R	huntingtin interacting protein-1-related
719	209590_at	AL157414		clone RP11-560A15 on chrom 20
720	209602_s_at	AI796169		GATA-binding protein 3
721	209603_at	AI796169		GATA-binding protein 3
722	209604_s_at	BC003070		GATA-binding protein 3, clone MGC:2346
723	209605_at	D87292	rhodanese	thiosulfate sulfurtransferase
724	209656_s_at	AL136550		DKFZp761J17121
725	209679_s_at	BC003379		hyp protein from clone 643, clone MGC:5115
726	209684_at	AL136924		DKFZp586G2120
727	209687_at	U19495	hIRH	intercrine-alpha
728	209691_s_at	BC003541	FLJ10488	hypothetical protein FLJ10488
729	209699_x_at	U05598		dihydrodiol dehydrogenase
730	209732_at	NM_005127		Sim to C-type lectin, superfam mem 2
731	209763_at	AL049176		clone 141H5 on chrom Xq22.1-23
732	209771_x_at	AA761181		CD24 antigen

733	209863_s_at	AF091627		CUSP
734	209866_s_at	R50822		KIAA0768 protein
735	209975_at	AF182276	CYP2E1	cytochrome P450-2E1
736	210059_s_at	BC000433		mitogen-activated protein kinase 13
737	210096_at	J02871		lung cytochrome P450 BI
738	210128_s_at	U41070		P2 purinergic receptor
739	210298_x_at	AF098518	FHL1	4 and ½ LIM domains 1 protein isoform B
740	210347_s_at	AF080216		C2H2-type zinc-finger protein
741	210372_s_at	AF208012	TPD52L2	tumor protein D52-like 2
742	210397_at	U73945		beta-defensin-1
743	210619_s_at	AF173154	HYAL1	hyaluronoglucosaminidase 1 isof 2
744	210633_x_at	M19156	KRT10	acidic keratin-10
745	210715_s_at	AF027205	kop	Kunitz-type protease inhibitor
746	210880_s_at	AB001467	Efs2	
747	210958_s_at	BC003646		clone MGC:4693
748	211043_s_at	BC006332	Lcb	clathrin, light polypeptide
749	211105_s_at	U80918	NF-ATcC	transcription factor
750	211382_s_at	AF220152	TACC2	transforming, acidic coiled-coil containing protein 2
751	211458_s_at	AF180519		GABA-A receptor-associated
752	211596_s_at	AB050468		membrane glycoprotein LIG-1
753	211597_s_at	AB059408	SMAP31-12	
754	211653_x_at	M33376		pseudo-chlordecone reductase
755	211712_s_at	BC005830		clone MGC:1925
756	211734_s_at	BC005912		Fc fragment IgE, high affinity I, rec for α polypep
757	211841_s_at	U94510		lymphocyte associated receptor of death 9, alternatively spliced
758	211986_at	BG287862	desmoyokin	AHNAK nucleoprotein
759	212148_at	BF967998		FLJ12900 fis,
760	212204_at	AF132733		DKFZP564G2022 protein
761	212242_at	AL565074		tubulin, alpha 1 (testis specific)
762	212327_at	AK027231		FLJ23578 fis, KIAA1102 protein
763	212328_at	AK027231		FLJ23578 fis, KIAA1102 protein
764	212390_at	AB007923		KIAA0477 gene product
765	212538_at	AL576253		KIAA1058 protein
766	212543_at	U83115.1		non-lens β gamma-crystallin like
767	212589_at	BG168858		oncogene TC21
768	212593_s_at	N92498		FLJ22071 fis, clone HEP11691
769	212724_at	BG054844		ras homolog gene family, member E
770	212741_at	AA923354		monoamine oxidase A
771	212823_s_at	AU147160		KIAA0599 protein
772	212841_s_at	AI692180		PTPRF interacting protein, binding protein 2
773	212850_s_at	AA584297		low density lipoprotein receptor-related protein 4
774	212875_s_at	AP001745		chrom 21 open reading frame 25
775	212992_at	AI935123		ESTs
776	213029_at	AL110126		DKFZp564H1916
777	213032_at	AL110126		DKFZp564H1916
778	213050_at	AA594937		KIAA0633 protein
779	213068_at	AI146848		dermatopontin
780	213071_at	AI146848		dermatopontin

781	213106_at	AI769688		23664 and 23905 mRNA sequence
782	213110_s_at	AW052179		collagen, type IV, alpha 5
783	213122_at	AI096375		KIAA1750 protein, partial cds
784	213135_at	U90902		clone 23612
785	213194_at	BF059159		Hs.301198 roundabout homolog
786	213227_at	BE879873		progesterone membrane binding
787	213280_at	AK000478		FLJ20471 fis
788	213285_at	AV691491		DKFZp564D1462
789	213287_s_at	X14487		acidic (type I) cytokeratin 10
790	213353_at	BF693921		ATP-binding cassette, sub-family A, member 5
791	213359_at	W74620		heterogeneous nuclear ribonucleoprotein D
792	213369_at	AI825832		DKFZp434A132
793	213375_s_at	N80918		Novel gene mapping to chomo 13
794	213397_x_at	AI761728		DnaJ homolog, subfam C, mem 8
795	213451_x_at	BE044614		tenascin XB
796	213456_at	AI927000		DKFZP564D206
797	213506_at	BE965369		proteinase activated receptor-2
798	213556_at	BE673445		chromosome 19, cosmid R28379
799	213618_at	AB011152		KIAA0580
800	213695_at	L48516	PON3	paraoxonase 3
801	213707_s_at	NM_005221	DLX5	distal-less homeo box 5
802	213725_x_at	AI693140		DKFZp586F071
803	213737_x_at	AI620911		
804	213800_at	X04697		complement factor H 38-kDa N-term
805	213817_at	AL049435		DKFZp586B0220
806	213820_s_at	T54159		hypothetical protein MGC10327
807	213844_at	NM_019102	HOXA5	homeo box A5
808	213848_at	AI655015		DKFZp586F2224
809	213891_s_at	AI927067		FLJ11918 fis
810	213924_at	BF476502		hypothetical protein FLJ11585
811	213929_at	AL050204		DKFZp586F1223
812	213933_at	AW242315		DKFZp586M0723
813	213935_at	AF007132		clone 23551
814	213942_at	AL134303		DKFZp547K034_r1
815	213992_at	AI889941		collagen, type IV, alpha 6
816	213994_s_at	AI885290		spondin 1, extracellular matrix
817	214058_at	M19720		L-myc protein
818	214132_at	BG232034		ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
819	214164_x_at	BF752277		adaptor-related protein complex 1, gamma 1 subunit
820	214234_s_at	X90579		cyp related pseudogene
821	214235_at	X90579		cyp related pseudogene
822	214247_s_at	AU148057		regulated in glioma
823	214598_at	AL049977		DKFZp564C122
824	214696_at	AF070569		clone 24659
825	214753_at	AW084068		BRCA2 region
826	214823_at	AF033199		C2H2 zinc finger protein pseudogene
827	215034_s_at	AI189753		FLJ13302 fis
828	215062_at	AL390143		DKFZp547N074
829	215129_at	AJ000008		C2 domain containing PI3-kinase

830	215239_x_at	AU132789		zinc finger protein 273
831	215243_s_at	AF099730	GJB3	connexin 31
832	215388_s_at	X56210	FHR-1	complement Factor H-related 1
833	215513_at	AF241534	HYMAI	hydatidiform mole assoc & imprinted
834	215516_at	AC005048		BAC clone CTB-15P3 fr 7q22-q31.2
835	215536_at			DMA, DMB, HLA-Z1, IPP2, LMP2, TAP1, LMP7, TAP2, DOB, DQB2 and RING8, 9, 13 and 14 genes
836	215659_at	AK025174		FLJ21521
837	215704_at	AL356504		clone RP1-14N1 chrom 1q21.1-21.3
838	215726_s_at	M22976		cytochrome b5
839	215867_x_at	AL050025		DKFZp564D066
840	216199_s_at	AL109942		clone RP3-473J16 chrom 6q25.3-26
841	216268_s_at	U77914		soluble protein Jagged
842	216333_x_at	M25813		unidentified gene complementary to P450c21
843	216379_x_at	AK000168		CD24 signal transducer
844	216594_x_at	S68290		chlordecone reductase homolog
845	216699_s_at	L10038		pre-pro-protein for kallikrein
846	217087_at	AF005081	xp32	skin-specific protein
847	217528_at	BF003134		ESTs
848	217707_x_at	AI535683		ESTs
849	217901_at	BF031829		desmoglein 2
850	217961_at	NM_017875	FLJ20551	hypothetical protein FLJ20551
851	218002_s_at	NM_004887	SCYB14	small inducible cytokine subfamily B (Cys-X-Cys), member 14
852	218170_at	NM_016048	LOC51015	CGI-111 protein
853	218180_s_at	NM_022772	FLJ21935	hypothetical protein FLJ21935
854	218186_at	NM_020387	CATX-8	CATX-8 protein
855	218237_s_at	NM_030674	ATA1	amino acid transporter system A1
856	218326_s_at	NM_018490	GPR48	G protein-coupled receptor 48
857	218434_s_at	NM_023928		hypothetical protein FLJ12389
858	218451_at	NM_022842		hypothetical protein FLJ22969
859	218499_at	NM_016542	LOC51765	serinethreonine protein kinase MASK
860	218546_at	NM_024709		hypothetical protein FLJ14146
861	218552_at	NM_018281		hypothetical protein FLJ10948
862	218603_at	NM_016217	LOC51696	hHDC for homolog of Dros headcase
863	218644_at	NM_016445	PLEK2	pleckstrin 2 (mouse) homolog
864	218651_s_at	NM_018357		hypothetical protein FLJ11196
865	218657_at	NM_016339	LOC51195	Link guanine nucleotide exchange factor II
866	218675_at	NM_020372	LOC57100	organic cation transporter
867	218677_at	NM_020672	LOC57402	S100-type calcium binding protein A14
868	218692_at	NM_017786		hypothetical protein FLJ20366
869	218704_at	NM_017763		hypothetical protein FLJ20315
870	218718_at	NM_016205	PDGFC	platelet derived growth factor C
871	218736_s_at	NM_017734		hypothetical protein FLJ20271
872	218751_s_at	NM_018315		hypothetical protein FLJ11071
873	218764_at	NM_024064		hypothetical protein MGC5363
874	218792_s_at	NM_017688		hypothetical protein FLJ20150
875	218796_at	NM_017671		hypothetical protein FLJ20116
876	218804_at	NM_018043		hypothetical protein FLJ10261
877	218806_s_at	AF118887	VAV-3	VAV-3 protein
878	218807_at	NM_006113	VAV3	vav 3 oncogene

879	218816_at	NM_018214		hypothetical protein FLJ10775
880	218820_at	NM_020215		hypothetical protein DKFZp761F2014
881	218849_s_at	NM_006663	RAI	RelA-associated inhibitor
882	218854_at	NM_013352	SART-2	squamous cell carcinoma antigen recog by T cell
883	218901_at	NM_020353	LOC57088	phospholipid scramblase 4
884	218919_at	NM_024699		hypothetical protein FLJ14007
885	218963_s_at	NM_015515		DKFZP434G032 protein
886	219010_at	NM_018265		hypothetical protein FLJ10901
887	219054_at	NM_024563		hypothetical protein FLJ14054
888	219064_at	NM_030569		hypothetical protein MGC10848
889	219073_s_at	NM_017784		hypothetical protein FLJ20363
890	219090_at	NM_020689	NCKX3	sodium calcium exchanger
891	219093_at	NM_017933		hypothetical protein FLJ20701
892	219095_at	NM_005090	PLA2G4B	phospholipase A2, group IVB
893	219109_at	NM_024532		hypothetical protein FLJ22724
894	219115_s_at	NM_014432	IL20RA	interleukin 20 receptor, alpha
895	219229_at	NM_013272	SLC21A11	solute carrier family 21, member 11
896	219232_s_at	NM_022073		hypothetical protein FLJ21620
897	219263_at	NM_024539		hypothetical protein FLJ23516
898	219298_at	NM_024693		hypothetical protein FLJ20909
899	219313_at	NM_017577		hypothetical protein DKFZp434C0328
900	219368_at	NM_021963	NAP1L2	nucleosome assembly protein 1-like 2
901	219388_at	NM_024915		hypothetical protein FLJ13782
902	219395_at	NM_024939		hypothetical protein FLJ21918
903	219410_at	NM_018004		hypothetical protein FLJ10134
904	219411_at	NM_024712		hypothetical protein FLJ13824
905	219423_x_at	NM_003790	TNFRSF12	TNF receptor superfamily, member 12
906	219436_s_at	NM_016242	LOC51705	endomucin-2
907	219461_at	AJ236915		pak5 protein
908	219476_at	NM_024115		hypothetical protein MGC4309
909	219489_s_at	NM_017821		hypothetical protein FLJ20435
910	219497_s_at	NM_022893	BCL11A	B-cell CLLlymphoma 11A
911	219518_s_at	NM_025165		hypothetical protein FLJ22637
912	219528_s_at	NM_022898	BCL11B	B-cell lymphomaleukaemia 11B
913	219532_at	NM_022726	ELOVL4	Stargardt disease 3
914	219597_s_at	NM_017434	DUOX1	dual oxidase 1
915	219689_at	NM_020163	LOC56920	semaphorin sem2
916	219729_at	NM_016307	PRX2	paired related homeobox protein
917	219764_at	NM_007197	FZD10	frizzled (Drosophila) homolog 10
918	219806_s_at	NM_020179	FN5	FN5 protein
919	219825_at	NM_019885	P450RAI-2	cyt P450 retinoid metabolizing
920	219908_at	NM_014421	DKK2	dickkopf homolog 2
921	219936_s_at	NM_023915	GPR87	G protein-coupled receptor 87
922	219938_s_at	NM_024430	PSTPIP2	proline-serine-threonine phosphatase interacting protein 2
923	219970_at	NM_017655		hypothetical protein FLJ20075
924	219976_at	NM_015888	HOOK1	hook1 protein
925	219995_s_at	NM_024702		hypothetical protein FLJ13841
926	219998_at	NM_014181		HSPC159 protein
927	220016_at	NM_024060		hypothetical protein MGC5395
928	220056_at	NM_021258	IL22R	interleukin 22 receptor
929	220066_at	NM_022162	NOD2	NOD2 protein
930	220076_at	NM_019847	ANKH	ankylosis, progressive homolog

931	220161_s_at	NM_019114	EHM2	EHM2 gene
932	220225_at	NM_016358	IRX4	iroquois homeobox protein 4
933	220230_s_at	NM_016229	LOC51700	cytochrome b5 reductase b5R.2
934	220262_s_at	NM_023932		hypothetical protein MGC2487
935	220266_s_at	NM_004235	KLF4	Kruppel-like factor 4 (gut)
936	220289_s_at	NM_017977		hypothetical protein FLJ10040
937	220318_at	NM_017957	FLJ20778	epsin 3
938	220413_at	NM_014579	ZIP2	zinc transporter
939	220414_at	NM_017422	CLSP	calmodulin-like skin protein
940	220428_at	NM_015717	LANGERIN	Langerhans cell specific c-type lectin
941	220432_s_at	NM_016593	CYP39A1	oxysterol 7alpha-hydroxylase
942	220518_at	NM_024801		hypothetical protein FLJ21551
943	220625_s_at	AF115403		Ets transcription factor ESE-2b
944	220723_s_at	NM_025087		hypothetical protein FLJ21511
945	220724_at	NM_025087		hypothetical protein FLJ21511
946	220911_s_at	NM_025081		KIAA1305 protein
947	220945_x_at	NM_018050		hypothetical protein FLJ10298
948	221127_s_at	NM_006394	RIG	regulated in glioma
949	221215_s_at	NM_020639	ANKRD3	ankyrin repeat domain 3
950	221541_at	AL136861		DKFZp434B044
951	221667_s_at	AF133207		protein kinase H11
952	221747_at	AL046979		DKFZp586K0617
953	221748_s_at	AL046979		DKFZp586K0617
954	221760_at	BG287153		mannosidase, α, class 1A, member 1
955	221796_at	AA707199		Similar to hyp protein FLJ20093
956	221841_s_at	BF514079		Kruppel-like factor 4 (gut)
957	221854_at	AI378979		ESTs
958	221922_at	AW195581		KIAA0761
959	221950_at	AI478455		empty spiracles homolog 2
960	222043_at	AI982754		clusterin
961	222102_at	NM_000847	GSTA3	glutathione S-transferase A3
962	222236_s_at	AK000253		FLJ20246 fis
963	222256_s_at	AK000550		FLJ20543 fis
964	222288_at	AI004009		ESTs
965	222290_at	AA731709		ESTs
966	222303_at	AV700891		ESTs
967	266_s_at	L33930		CD24 signal transducer
968	33322_i_at	X57348		clone 9112
969	33323_r_at	X57348		clone 9112
970	35666_at	U38276		semaphorin III family homolog
971	38340_at	AB014555		KIAA0655 protein
972	39248_at	N74607		za55a01.s1
973	40016_g_at	AB002301		KIAA0303 gene
974	40093_at	X83425		LU gene Lutheran blood group glycoprotein
975	40472_at	AF007155		clone 23763 unknown mRNA
976	57588_at	R62432		yg52e11.s1
977	60474_at	AA469071		ne17f11.s1
978	91826_at	AI219073		qg16e08.x1
979			PBGD	
980			MART1	
981			Me20m	
982			MAGE-3	
983				Me20m forward primer

984				Me20m reverse primer
985				Me20m probe
986				PBGD forward primer
987				PBGD reverse primer
988				PBGD probe
999				Tyrosinase
1000				Tyrosinase Forward
1001				Tyrosinase Reverse
1002				Tyrosinase probe
1003				MART1 Forward
1004				MART1 Reverse
1005				MART1 Probe
1006				HMB45 Forward
1007				gp100 Reverse
1008				gp100 Probe
1009				PLAB Forward
1010				PLAB Reverse
1011				PLAB Probe

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